

## REMARKS

Applicants submit herewith a replacement sheet for sheet 5 containing Figures 9A and 9B. As shown in the annotated sheet 5 submitted herewith, the nucleotide sequence of the VL domain of 7F3com-2H2 has been amended to correct an error in the sequence. Specifically, nucleic acid residue 202 in framework region of the VL domain has been corrected in Figure 9B. Applicants respectfully assert that support for the correct nucleotide sequence is found in the ATCC deposit of 7F3com-2H2. The deposit of a nucleotide sequence, such as the nucleotide sequence of the 7F3com-2H2 antibody deposited with ATCC, provides sufficient written description of the nucleotide sequence. *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002).

Applicants respectfully point out that one of skill in the art would appreciate that the nucleotide at position 202 in Figure 9B is an error since the codon composed of the nucleotide at position 202 encodes a tyrosine residue at position 67 of the amino acid sequence of the VL domain of the 7F3com-2H2 antibody as opposed to the serine residue recited in Figure 8B and in paragraph [0184] of the specification as filed. Thus, one of skill would look to ATCC deposit No. PTA-5913 for the correct nucleotide sequence at position 202 of the VL domain of 7F3com-2H2. Accordingly, the amendment to Figure 9B does not constitute new matter. Entry of replacement sheet into the record of the present application is respectfully requested.

Applicants submit herewith, in compliance with 37 C.F.R. §§ 1.121(a)(3) and 1.125(b) and (c), a Substitute Specification and a marked-up version of the Substitute Specification showing all the changes made relative to the specification as originally filed. The Substitute Specification corrects the sequence identifiers assigned to the complementarily determining regions (CDRs) in Table 1 and the references to those sequence identifiers in the specification. In some instances, the specification as filed assigned the same sequence identifier to two different sequences in Table 1. The Substitute Specification corrects this error by assigning two different sequence identifiers to two different sequences assigned the same sequence identifier in the specification as filed. For example, Table 1 in the specification as filed assigned SEQ ID NO:2 to the amino acid sequence of the VH CDR3 of the 4D4 antibody and the amino acid sequence of the VH CDR3 of the 71A10 antibody. In the Substitute Specification, the amino acid sequence of the VH CDR3 of the 4D4 antibody is assigned SEQ ID NO:61 and the amino acid sequence of the VH CDR3 of the 71A10 antibody is assigned SEQ ID NO.2.

The specification has also been amended to correct typographical errors in paragraph [0175] of the specification as originally filed. Specifically, the second sentence has been amended in the Substitute Specification to refer to VH CDRs instead of VL CDRs. One of skill in the art would recognize that Applicants meant one of the two sentences in paragraph [0175] to recite VH CDRs instead of VL CDRs since the two sentences in the specification as filed are identical.

Applicants respectfully assert that the amendments to the specification set forth in the Substitute Specification are to correct errors obvious to one skilled in the art and do not constitute new matter. Accordingly, entry of the Substitute Specification into the record of the application is respectfully requested.

Applicants also submit herewith a Substitute Sequence Listing. The Substitute Sequence Listing includes the CDRs recited in Table 1 that were incorrectly assigned the same sequence identifier in the specification as originally filed. The Substitute Sequence Listing also corrects the error in nucleotide 202 of VL domain of 7F3com-2H2.

Claims 1-110 have been canceled without prejudice. Applicants reserve the right to pursue the subject matter of the canceled claims in a related application. New claims 111-168 have been added. Support for the newly added claims may be found throughout the specification as filed and, for example, as provided in the table below.

<b>Claim(s)</b>	<b>Support in the Specification [paragraph no.]</b>
111	66, 111, 544
112	66, 111, 544, 139 (FIGS. 8A-8B), Table 1
113	66, 111, 544, 151, Table 1
114	66, 111, 544, 153, Table 1
115	66, 111, 544, 152, 153, Table 1
116, 120, 121, 122	66, 111, 544, 152, 153, Table 1, 157
117, 118, 119, 123, 124, 125, 126	66, 111, 544, 152, 153, Table 1, 151
127	66, 111, 544, 152, 153, Table 1, 151, 84
128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142	66, 11, 139 (FIGS. 8A-8B), Table 1
143	66, 111, 544, 151, Table 1
144	66, 111, 544, 153, Table 1
145, 146, 147	66, 111, 544, 151, Table 1, 155

Claim(s)	Support in the Specification [paragraph no.]
148	207
149	208
150, 151	66, 11, 139 (FIGS. 8A-8B), Table 1, 155
152	84
153	84
154	544
155	221, 225
156	221, 224
157	381, 382
158	381, 382, 385
159	387, 388
160	398, 399
161	393
162	480, 481
163-167	155, 169
168	377

Applicants respectfully request that the amendments and remarks made herein be entered and fully considered. Claims 111-168 are currently pending in the present application. No new matter has been introduced.

**I. STATEMENT OF SUBSTANCE OF INTERVIEW UNDER 37 C.F.R. § 1.133**

Applicants thank Examiner Jo Ann Rinaudo and Supervisory Patent Examiner Christina Chan for the courtesy of the telephonic interview on February 23, 2006.

Pursuant to 37 C.F.R. § 1.133 and MPEP § 713.04, and in connection with the Office Action mailed November 29, 2005, Attorneys for Applicants submit this Statement of Substance of Interview in connection with the telephonic interview of February 23, 2006 between Examiner Jo Ann Rinaudo and Supervisory Patent Examiner Christina Chan, and Attorneys for Applicants, Margaret Brivanlou, Jennifer Chheda, and Lynda Nguyen.

During the telephonic interview of February 23, 2006, the enablement and written description rejections under 35 U.S.C. § 112, first paragraph, were discussed. Specifically discussed were the lack of enablement and lack of written description rejections

for claims directed to an antibody comprising less than 6 complementarity determining regions (“CDRs”) that immunospecifically bind to an IL-9 polypeptide. Attorneys for Applicants argued that the present application provides enabling disclosure and sufficient written description support for such an antibody. Attorneys for Applicants discussed references available to one of skill in the art as of the effective filing date of the application to show that antibodies comprising less than 6 CDRs were known in the art. In particular, Attorneys for Applicants discussed references that disclosed single domain antibodies comprising only a VH domain or a VL domain with 3 CDRs that are able to bind to antigens with high affinity and specificity. Examiner Chan stated that she would consider such references if cited by Applicants, and would discuss this issue with other Examiners. Attorneys for Applicants stated that they would provide such references with their Reply to the Office Action. Examiner Chan stated that claims to an antibody comprising a VH domain or a VL domain with 3 specified CDRs would be acceptable.

**II. THE REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH, SHOULD BE WITHDRAWN**

Claims 1-87 are rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner states that the use of “7F3com-2H2” renders the claims indefinite because such term is a laboratory designation and because different laboratories may use the same designation to define completely distinct laboratory materials. The Examiner suggests that Applicants amend the claims to recite the accession number of the deposited *E. coli* containing the pMI347 vector encoding the anti-IL-9 antibody 7F3com-2H2. Office Action at p. 3.

Applicants respectfully disagree with the rejection. The specification clearly sets forth the amino acid sequences of the antibody designated 7F3com-2H2. See, *e.g.*, Table 1 and Figures 8A-8B, which set forth the amino acid sequences of the VH domain, VH CDR1, VH CDR2, VHCDR3, VL domain, VL CDR1, VL CDR2, and VL CDR3 of 7F3com-2H2. Therefore, Applicants respectfully assert that the reference to the antibody 7F3com-2H2 in the claims is not indefinite and that one of skill in the art would be able to ascertain what is meant by the term “7F3com-2H2.”

However, in order to expedite prosecution of the application and without conceding to the propriety of the rejection, Applicants have canceled claims 1-87 and added new claims 111-168. New independent claims 111, 115, 119, 123, 143, 144, 145 and 154



(and claims dependent thereon) recite ATCC deposit number PTA-5913 to refer to the 7F3com-2H2 anti-IL-9 antibody as encoded by the pMI347 expression vector contained in the *E. coli* DH $\alpha$  K12 microorganism deposited with the American Type Culture Collection (ATCC), located in Manassas, Virginia, on April 9, 2004 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure ("Budapest Treaty"). Accordingly, the rejection of claims 1-87 for indefiniteness has been obviated, and Applicants respectfully request that the rejection be withdrawn.

### **III. THE REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, FOR LACK OF ENABLEMENT SHOULD BE WITHDRAWN**

Claims 1-87, 101 and 102 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly not containing subject matter which was described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. For the reasons detailed below, the rejections under 35 U.S.C. § 112, first paragraph, for lack of enablement cannot stand and should be withdrawn.

#### **A. Availability of ATCC Deposit**

The Examiner contends that an affidavit or declaration by Applicants, or a statement by an attorney of record is required to assure that the deposited material recited in claims 1-87, 101 and 102 (canceled; presently recited in new claims 111, 115, 119, 123, 143, 144, 145 and 154, and dependent claims thereon) will be irrevocably and without restriction released to the public upon the issuance of a patent. Office Action at pp. 3-4. Applicants respectfully direct the Examiner's attention to the attached Statement of Attorneys for Applicants Regarding the Permanence and Availability of Deposited Microorganisms ("Statement"; Exhibit 1), which attests to the deposit of microorganisms according to the provisions of the Budapest Treaty, in compliance with the criteria set forth by 37 C.F.R. §§ 1.801-1.809 regarding the availability and permanence of deposits. Accordingly, the Statement obviates the Examiner's rejection based on the availability of the deposited material recited in new claims 111, 115, 119, 123, 143, 144, 145, and 154, and dependent claims thereon.

**B. The Specification Enables the Claimed Invention**

The Examiner contends that the specification does not reasonably provide enablement for an anti-IL-9 antibody comprising any number less than 6 CDRs. Office Action at p. 4. Relying on Janeway et al., *Immunobiology*, 6<sup>th</sup> ed., 2005, Garland Publishing, pp. 110-112 (“Janeway”), the Examiner contends that all of the heavy chain and light chain CDRs as well as the framework regions are required in order to form functional antigen binding sites. Office Action at p. 5. The Examiner further cites Rudikoff et al., 1982, *Proc. Natl. Acad. Sci. USA* 79:1979-1983 (“Rudikoff”) for the contention that alteration of a single amino acid in a CDR could dramatically affect antigen binding. Office Action at p. 5. Applicants respectfully disagree for the reasons provided below.

Claims 1-87, 101 and 102 have been canceled without prejudice to Applicants’ right to pursue the subject matter of the canceled claims in a related application(s). New claims 111-168 have been added. Independent claim 111 (and claims dependent therefrom) recites an isolated antibody comprising a CDR of the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Independent claim 115 (and claims dependent therefrom) recites an isolated antibody comprising two CDRs of the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Independent claim 119 (and claims dependent therefrom) recites an isolated antibody comprising three CDRs of the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Independent claim 123 (and claims dependent therefrom) recites an isolated antibody comprising four CDRs of the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide.

Independent claim 128 (and claims dependent therefrom) recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, wherein the antibody comprises the 3 CDRs of the VH domain of the 7F3com-2H2 antibody. Independent claim 135 (and claims dependent therefrom) recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, wherein the antibody comprises the 3 CDRs of the VL domain of the 7F3com-2H2 antibody. Dependent claim 142 recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, wherein the antibody comprises the 3 CDRs of the VH domain and the 3 CDRs of the VL domain of the 7F3com-2H2 antibody.

Thus, claim 142 recites an antibody specific for a human IL-9 polypeptide that is composed of 6 specified CDRs.

Independent claim 143 (and claims dependent therefrom) recites an isolated antibody comprising the VH domain of the antibody encoded by the vector as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Independent claim 144 (and claims dependent therefrom) recites an isolated antibody comprising the VL domain of the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Dependent claim 145 recites an isolated antibody comprising the VH and VL domains of the antibody encoded by the vector deposited as ATCC deposit No. 5913, wherein the antibody immunospecifically binds to a human IL-9 polypeptide. Thus, claim 145 recites an antibody specific for a human IL-9 polypeptide that is composed of 6 specified CDRs and the framework regions between the CDRs.

Independent claim 154 (and claims dependent therefrom) recites an isolated the antibody encoded by the vector deposited as ATCC deposit No. PTA-5913; i.e., an antibody composed of 6 CDRs. Independent claim 163 (and claims dependent therefrom) recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, the antibody comprising the VH CDRs of the 7F3com-2H2 antibody with each CDR having less than 4 amino substitutions. Independent claim 164 (and claims dependent therefrom) recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, the antibody comprising the VL CDRs of the 7F3com-2H2 antibody with each CDR having less than 4 amino acid substitutions. Dependent claim 167 recites an isolated antibody that immunospecifically binds to a human IL-9 polypeptide, the antibody comprising the VH and VL CDRs of the 7F3com-2H2 antibody with each CDR having less 4 amino acid substitutions.

Thus, the rejection of claims 1-87 and 101 and 102 under 35 U.S.C. § 112, first paragraph, for lack of enablement has been obviated. For the reasons provided below, the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement should not be applied to the new claims.

The legal test for enablement is “whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation.” MPEP § 2164.01, Rev. 3, August 2005; and *U.S. v. Teletronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988). Enablement is not precluded even if some experimentation is necessary. *Hybritech, Inc. v. Monoclonal*

*Antibodies, Inc.*, 802 F.2d 1367 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). The Court of Appeals for the Federal Circuit has determined that experimentation, though laborious, is not undue experimentation where the specification provides a reasonable amount of guidance. *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). Moreover, a “patent need not teach, and preferably omits, what is well known in the art.” *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). For the reasons detailed below, the specification provides one of ordinary skill in the art with sufficient guidance to meet the requirements of Section 112, first paragraph.

Applicants respectfully submit that the Examiner has improperly relied on the generic teaching in Janeway regarding antibodies to limit an antibody with antigen-binding activity to an antibody composed of “all of the heavy and light chain CDRs in their proper order and in the context of framework sequences which maintain their required conformation.” Office Action at page 5. The excerpt from Janeway cited by the Examiner is a general teaching of the structure of the antigen-binding site of a classic IgG antibody molecule, wherein it is taught that the six hypervariable loops, commonly termed the complementarity-determining regions, or CDRs, determine antigen specificity. Janeway at pp. 110-111. Janeway teaches that there are three CDRs located in each of the heavy and light chains, namely, CDR1, CDR2 and CDR3. These CDRs are juxtaposed between less variable domains known as the framework regions, which are commonly designated as FR1, FR2, FR3 and FR4, which are thought to provide the structural framework for the CDRs. Janeway at p. 111. Applicants do not disagree that the classic structure of the antigen-binding domain of an IgG antibody that Janeway teaches is composed of 6 CDRs. Rather, Applicants respectfully point out that an antibody does not require all of the CDRs from both the heavy chain and light chain for antigen binding, as the Examiner contends. Indeed, as taught in the specification (see, *e.g.*, paragraphs [0084] and [0503] of the specification of the present application and references disclosed therein) and commonly known by one of skill in the art, an antibody molecule can be composed of less than all 6 CDRs and still have high affinity and specificity for a particular antigen.

The specification teaches methods for producing antibodies comprising less than 6 CDRs with specificity for a particular antigen. In particular, the specification teaches single domain antibodies, *i.e.*, antibodies composed of either 3 CDRs of the VH domain or the VL domain (see, *e.g.*, paragraphs [0084] and [0503] of the specification of the present application). The specification also teaches methods for identifying those antibodies that immunospecifically bind to a human IL-9 polypeptide and methods for determining the

binding affinity of antibodies for a human IL-9 polypeptide (see, *e.g.*, Section 5.6.1 of the specification, in particular paragraphs [0434] and [0435] of the specification of the present application). Further, the specification provides *in vitro* and *in vivo* methods for determining the ability of such antibodies to modulate a biological activity associated with IL-9 (see Sections 5.6.2 and 5.6.3 of the specification of the present application).

The prior art is replete with examples of antibody molecules composed of less than 6 CDRs that have high affinity and specificity for a particular antigen. As of the effective filing date of the present application, methods for producing, for example, single domain antibodies composed of a single variable heavy (VH) domain or a single variable light (VL) domain with high antigen affinity and specificity for a particular antigen were known in the art. The specification teaches that single domain antibodies could be produced by methods well-known in the art (see, *e.g.*, paragraph [0503] of the specification of the present application) and cites a number of references for such methods. In addition to the particular references cited in the specification for single domain antibodies and methods for producing such antibodies, numerous other references were available as of effective date of the present application, some of which are discussed herein.

For example, Ward et al., 1989, *Nature* 341:544-546 (“Ward”; cited as reference C33 in the accompanying Supplemental Information Disclosure Statement (“IDS”)) teaches that single domain antibodies (“dAbs”) composed of only a VH domain with 3 CDRs may be prepared, and that such dAbs can have good antigen binding affinities. Further, Ward teaches that the dAbs “can be generated in a matter of days without recourse to tissue culture, and may also have other advantages over monoclonal antibodies,” *e.g.*, for tissue penetration due to their small size. Ward at p. 546.

Dumoulin et al., 2002, *Protein Sci.* 11:500-515 (“Dumoulin”; reference C17 in the accompanying Supplemental IDS) teaches that single domain antibodies comprising only VH domains derived from llamas, can be isolated by biopanning from phage libraries containing the genes coding for the variable domains of the heavy chain antibodies. Such antibodies can be expressed in *E. coli* and purified to produce highly stable, soluble antibodies with high affinity and specificity for binding to particular antigens. Dumoulin at pp. 501-502 and 511-512.

Davies et al., 1995, *Bio/Technol.* 13:475-479 (“Davies”; reference C16 in the accompanying Supplemental IDS) teaches that single domain antibodies containing only VH domains with 3 CDRs, can be isolated from a phage displayed VH library and expressed in *E. coli*. Such dAbs are highly specific, well expressed, stable and highly expressed in the *E.*

*coli*. Moreover, they displayed high affinity and specificity for antigen binding. Davies at pp. 476-477.

Van den Beucken et al., 2001, *J. Mol. Biol.* 310: 591-601 (“van den Beucken”; reference C32 in the accompanying Supplemental IDS) teaches that single domain antibodies composed of a VL domain with three CDRs may be generated using a phage-assisted protein engineering strategy, and that such single VL domain antibodies bind antigen with high affinity (*e.g.*, 191 nM) and specificity. van den Beucken at pp. 597-599.

Pereira et al., 1998, *Biochem.* 37:1430-1437 (“Pereira”; reference C26 in the accompanying Supplemental IDS) teaches that single domain antibodies containing only a VL domain with 3 CDRs can be produced using hybridoma-derived antibodies that are expressed as single chain variable fragments (scFv) in *E. coli*. These antibodies displayed high affinity and specificity for cardiolipin. Pereira at pp. 1434-1436.

Therefore, antibodies composed of less than 6 CDRs as well as methods for producing such antibodies were commonly known in the art as of the effective filing date of the present application and described in the specification of the present application.

The Examiner has cited Rudikoff, published over 20 years ago, for the proposition “that even minor changes in the amino acid sequence of the heavy and light variable regions, particularly in the CDRs, may dramatically affect the antigen-binding function.” Office Action at page 5. Applicants respectfully point out that the goal in Rudikoff was to identify antigen-binding variants that had less or no binding affinity to phosphocholine as compared to the parental antibody. Rudikoff was successful in identifying and characterizing a binding protein that had one amino acid substitution and had reduced affinity for binding the antigen, phosphocholine. Accordingly, Applicants concede that it may be possible that a substitution of one amino acid of a CDR may decrease or possibly ablate binding to the respective antigen. However, Applicants respectfully assert that antibodies that retain the ability to bind to the same antigen as the parental antibody could be produced without undue experimentation. Moreover, Applicants also respectfully submit that Rudikoff is a 1982 publication, and that the art of antibody engineering has progressed significantly since then. Further, as the references described above teach, one of skill in the art of antibody engineering would have been able to generate an antibody comprising less than 6 CDRs with high affinity and specificity for a particular antigen, without undue experimentation.

As of the effective filing date of the present application, methods for producing antibodies by combining one, two, three or more specific CDRs of an antibody

known to be specific for a particular antigen with other CDRs were known in the art. Applicants respectfully direct the Examiner's attention to the following prior art references which describe production of antibodies specific for a particular antigen by combining one or two CDRs from an antibody known to specifically bind to the antigen with other CDRs: Marks et al., 1992, *Bio/Technology* 10:779-783 ("Marks"; reference C22 in the accompanying Supplemental IDS); Klimka et al., 2000, *Brit. J. Cancer* 83(2):252-260 ("Klimka"; reference C19 in the accompanying Supplemental IDS); and Rader et al., 1998, *Proc. Natl. Acad. Sci. USA* 95:8910-8915 ("Rader"; reference C27 in the accompanying Supplemental IDS).

Marks teaches that antibodies with as much as 20-fold improved affinity could be generated by sequentially replacing (*i.e.*, shuffling) the heavy and light chain variable region genes with repertoires of variable region genes. More importantly, Marks teaches that the antibodies with improved affinity were produced by holding the third hypervariable loop, or the HCDR3, of the parental antibody constant while shuffling the remaining five CDRs.

Klimka teaches production of human recombinant antibodies by a technique involving guided phage antibody selection and cell panning, wherein only the HCDR3 is kept constant while the remaining five CDRs are varied. Klimka teaches that the human recombinant antibodies had the same specificity for antigen as the parental antibody.

Rader teaches the humanization of the mouse anti- $\alpha_v\beta_3$  antibody LM609 using phage-display, wherein HCDR3 and the LCDR3 were maintained and all the other four CDRs were replaced by human sequences selected from phage-displayed antibody libraries. The resultant humanized antibody variants had affinities as high or higher than the affinity of the original antibody.

Taken together, Marks, Klimka and Rader demonstrate that methods for producing antibodies with specificity for a particular antigen could be generated, as of the effective filing date of the application, by combining one or two specific CDRs from an antibody specific for the antigen with other unknown CDRs. Thus, one of skill in the art as of the effective filing date of the present application would have been able to produce antibodies composed of 6 CDRs or less than 6 CDRs which immunospecifically bind to a human IL-9 polypeptide, without undue experimentation, by combining one, two or more specific CDRs from the 7F3com-2H2 antibody with other CDRs (known or unknown). Therefore, Applicants respectfully submit that the specification of the present application fully enables one of skill in the art to produce the claimed antibodies, without undue experimentation.

In view of the foregoing the rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement cannot stand and should be withdrawn.

**IV. THE REJECTION UNDER 35 U.S.C. § 112,  
FIRST PARAGRAPH, FOR LACK OF LACK  
OF WRITTEN DESCRIPTION SHOULD BE WITHDRAWN**

Claims 1-87, 101 and 102 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the claimed invention. Office Action at p. 6. Specifically, the Examiner contends that there is insufficient written description for an antibody that immunospecifically binds to a human IL-9 polypeptide comprising less than 6 specific known CDR sequences, other than those provided by the amino acid sequences corresponding to the VH domain and VL domain of the antibody known as 7F3com-2H2. *Id.* For the reasons detailed below, the rejection cannot stand and should be withdrawn.

As discussed above, claims 1-87, 101 and 102 have been canceled and new claims 111-168 have been added. Thus, the rejection of claims 1-87, 101 and 102 under 35 U.S.C. § 112, first paragraph, for lack of written description has been obviated. For the reasons provided below, the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description should not be applied to the new claims.

The legal standard for written description requirement under Section 112, first paragraph, has been set forth by the Federal Circuit. *See University of Rochester v. G.D. Searle & CO., Inc.*, 358 F.3d 916, 921-23 (Fed. Cir. 2004); *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319-21 (Fed. Cir. 2003); *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1566-67 (Fed. Cir. 1997); and *Vas-Cath v. Mahurkar*, 935 F.2d 1555, 1560-64 (Fed. Cir. 1991). Indeed, much of this case law on the written description requirement has in large part been adopted in the Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, “Written Description” Requirement, Fed. Reg. Vol. 66, No. 4 at pp. 1099-1111 (January 5, 2001), hereinafter, “Written Description Guidelines”; and MPEP § 2163 (Rev. 3, Aug. 2005). To satisfy the written description requirement, an applicant must “convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention.” *Vas-Cath*, 935 F.2d at 1563-64 (emphasis in original) and MPEP § 2163 at p. 2100-172. Moreover, it is well established that the specification need not describe, and should preferably omit what is known in the art. *Hybritech Inc. v. Monoclonal Antibodies*,



Inc., 802 F.2d 1367 (Fed. Cir. 1986); *In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527 (Fed. Cir. 1992); and *Vas-Cath*, 935 F.2d 1555.

Applicants respectfully assert that the specification, coupled with information well-known in the art as of the effective filing date of the present application, would have reasonably conveyed to one of skill in the art, as of the effective filing date of the present application, that Applicants were in possession of an anti-IL-9 antibody comprising less than the 6 known CDR sequences of the 7F3com-2H2 antibody.

The specification of the present application provides antibodies that immunospecifically bind to an IL-9 polypeptide comprising less than the 6 known CDRs corresponding to those of 7F3com-2H2. Applicants respectfully direct the Examiner's attention to Section 5.1, entitled "IL-9 Antibodies," and specifically, paragraphs [0171] to [0182] and TABLE 1. Paragraph [0172], *e.g.*, states, in part:

The present invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 1, *infra*. In particular, the invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising (or alternatively, consisting of) one, two, three or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 1, *infra*.

There is similar disclosure for antibodies comprising a VL CDR having an amino acid sequence of any one of the VL CDRs provided in Table 1 (see paragraph [0174] of the specification of the present application). The specification teaches that a VH CDR(s) or a VL CDR(s) can be combined with a VH CDR(s) or a VL CDR(s), respectively, disclosed in U.S. provisional application Serial Nos. 60/371,683 and 60/371,728, both filed April 12, 2002 (see, *e.g.*, paragraph [0156] of the specification of the present application). The specification teaches that a CDR(s) of the 7F3com-2H2 antibody can be combined with one or more CDRs of the other antibodies disclosed in Table 1 (see, *e.g.*, paragraph [0157] of the specification of the present application). The specification also teaches that antibodies comprising less than 6 CDRs that immunospecifically bind to a human IL-9 polypeptide may be single domain antibodies and provides methods for producing such single domain antibodies (*e.g.*, paragraphs [0084] and [0503] of the specification of the present application). Further, as discussed above, methods for producing antibodies composed of less than 6 CDRs, in particular single domain antibodies, were known in the art.

Thus, as of the effective filing date of the present application, the specification coupled with the state of the art with respect to antibody engineering does, indeed, provide sufficient written description of the structural and functional features of the antibodies of the claimed invention to reasonably convey that Applicants were in possession of the claimed invention.

In view of the foregoing the rejection under 35 U.S.C. § 112, first paragraph, for lack of written description cannot stand and should be withdrawn.

**V. THE REJECTIONS UNDER 35 U.S.C. § 103(a) SHOULD BE WITHDRAWN**

Claims 107 and 108 are rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 6,261,559 in view of Liebman et al. Claims 103-110 are rejected as being unpatentable over U.S. Patent No. 6,261,559 in view of U.S. Patent Publication No. US 2002/0051787 A1. Claims 103-110 have been canceled without prejudice to Applicant's right to pursue the subject matter of the canceled claims in a related application(s). Accordingly, the Examiner's rejections under 35 U.S.C. § 103(a) are moot and should be withdrawn.

**CONCLUSION**

Applicants respectfully request that the above-made remarks and amendments be entered and made of record in the present application. If any issues remain in connection herewith, the Examiner is respectfully invited to telephone the undersigned to discuss the same. An early allowance is earnestly requested.

Respectfully submitted,

Date: May 1, 2006

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7F3com-2H2 V<sub>H</sub>

1	CAGGTGCAG	CTGGTGCAG	TCTGGGGCT	GAGGTGAAG	AAGCCTGGG
46	TCCTCAGTG	AAGGTTTCC	TGCAAGGCA	TCTGGAGGC	ACCTTCAGC
91	TATTACTGG	ATAGAGTGG	GTGCGACAG	GCCCCTGGA	CAAGGGCTT
136	GAGTGGATG	GGAGAGATT	TTACCTGGA	AGTGGTACT	ACTAACCCG
181	AATGAGAAG	TTCAAGGGC	AGAGTCACC	ATTACCGCG	GACGAATCC
226	ACGAGCACA	GCCTACATG	GAGCTGAGC	AGCCTGAGA	TCTGAGGAC
271	ACGGCCGTG	TATTACTGT	GCGAGAGCG	GATTACTAC	GGTAGTGAT
316	TACGTCAAG	TTTGACTAC	TGGGGCCAA	GGAACCCTG	GTCACCGTC
361	TCCTCA				

**Fig. 9A**7F3com-2H2 V<sub>L</sub>

1	GACATCCAG	ATGACCCAG	TCTCCATCC	TCCCTGTCT	GCATCTGTA
46	GGAGACAGA	GTCACCATC	ACTTGCAAG	GCAAGTCAG	CATGTGATT
91	ACTCATGTA	ACCTGGTAT	CAGCAGAAA	CCAGGGAAA	GCCCCTAAG
136	CTCCTGATC	TATGGGACA	TCCTACAGC	TACAGTGGG	GTCCCATCA
181	AGGTTCACT	GGCAGTGGA	TCTGGGACA	GATTTCACT	CTCACCATC
226	AGCAGTCTG	CAACCTGAA	GATTTTGCA	ACTTATTAC	TGTCAGCAA
271	TTTTACGAG	TATCCTCTC	ACGTTTCGGC	GGAGGGACC	AAGGTGGAG
316	ATCAAA				

↑  
"A" changed to "C"

**Fig. 9B**

**MARKED-UP SPECIFICATION**

**RECOMBINANT IL-9 ANTIBODIES AND USES THEREOF**

[001] This application claims priority to U.S. Provisional Application Serial No. 60/477,797, filed June 10, 2003, U.S. Provisional Application Serial No. 60/462,259, filed April 11, 2003, which are both incorporated by reference herein in their entireties.

**1. FIELD OF THE INVENTION**

[002] The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide and compositions comprising said antibodies. The invention also provides prophylactic and therapeutic protocols to prevent, treat, manage, and/or ameliorate various disorders or one or more symptoms thereof, said protocols comprising the administration of antibodies that immunospecifically bind to an IL-9 polypeptide alone or in combination with other therapies. In particular, the present invention provides methods for preventing, treating, managing, and/or ameliorating symptoms associated with an inflammatory disorder (*e.g.*, asthma) or a respiratory infection, said methods comprising administering to a human subject an effective amount of one or more antibodies thereof that immunospecifically bind to an IL-9 polypeptide. The present invention also provides pharmaceutical compositions comprising antibodies or fragments thereof that immunospecifically bind to an IL-9 polypeptide for the prevention, treatment, management, and/or amelioration of various disorders. The invention further provides methods for detecting or diagnosing IL-9 expression and disorders associated with aberrant IL-9 expression utilizing compositions comprising antibodies or fragments thereof that immunospecifically bind to an IL-9 polypeptide. The present invention further relates to articles of manufacture and kits comprising antibodies that immunospecifically bind to an IL-9 polypeptide for use in the prevention, management, treatment, and/or amelioration of various disorders.

**2. BACKGROUND OF THE INVENTION**

**2.1 AUTOIMMUNE DISORDERS**

[003] Autoimmune diseases are caused when the body's immune system, which is meant to defend the body against bacteria, viruses, and any other foreign product, malfunctions and produces antibodies against healthy tissue, cells and organs. Antibodies,

T cells and macrophages provide beneficial protection, but can also produce harmful or deadly immunological responses.

[004] The principle mechanisms by which auto-antibodies can produce an autoimmune disease are complement-dependent lytic destruction of the target cell, opsonization, formation of immune complexes, blockade of receptor sites for physiological ligands, and stimulation of cell surface receptors. The auto-antibody can bind to cell surface receptors and either inhibit or stimulate the specialized function of the cell (Paul, W.E. Ed., 1989, *Fundamental Immunology*, Raven Press, New York, Chapter 31, p. 839).

[005] Autoimmune diseases can be organ specific or systemic and are provoked by different pathogenic mechanisms. Organ specific autoimmunization is characterized by tolerance and suppression within the T cell compartment, aberrant expression of major-histocompatibility complex (MHC) antigens, antigenic mimicry and allelic variations in MHC genes. Systemic autoimmune diseases involve polyclonal B cell activation and abnormalities of immunoregulatory T cells, T cell receptors and MHC genes. Examples of organ specific autoimmune diseases are diabetes, Hashimoto's disease, autoimmune adrenal insufficiency, pure red cell anemia, multiple sclerosis and rheumatic carditis. Representative systemic autoimmune diseases are systemic lupus erythematosus, rheumatoid arthritis, chronic inflammation, Sjogren's syndrome polymyositis, dermatomyositis and scleroderma.

[006] Current treatment of autoimmune diseases involves administering immunosuppressive agents such as cortisone, aspirin derivatives, hydroxychloroquine, methotrexate, azathioprine and cyclophosphamide or combinations thereof. The dilemma faced when administering immunosuppressive agents, however, is the more effectively the autoimmune disease is treated, the more defenseless the patient is left to attack from infections. Thus, there is a need for an effective treatment for autoimmune diseases that does not compromise the patient's immune system.

## **2.2 INFLAMMATORY DISORDERS**

[007] Inflammation plays a fundamental role in host defenses and the progression of immune-mediated diseases. The inflammatory response is initiated by tissue injury (*e.g.*, trauma, ischemia, and foreign particles) and infection by a complex cascade of events, including chemical mediators (*e.g.*, cytokines and prostaglandins) and inflammatory cells (*e.g.*, leukocytes). The inflammatory response is characterized by increased blood flow,

increased capillary permeability, and influx of phagocytic cells. These events result in swelling, redness, warmth, and pus formation at the site of injury or infection.

**[008]** A delicate well-balanced interplay between the humoral and cellular immune elements in the inflammatory response enables the elimination of harmful agents and the initiation of the repair of damaged tissue. When this delicately balanced interplay is disrupted, the inflammatory response may result in considerable damage to normal tissue and may be more harmful than the original injury or infection that triggered the initial inflammatory reaction. In these cases of uncontrolled inflammatory responses, clinical intervention is needed to prevent tissue damage and organ dysfunction. Diseases and disorders such as rheumatoid arthritis, osteoarthritis, Crohn's disease, psoriasis, inflammatory bowel disease, asthma, and allergies are characterized by inflammation.

**[009]** Current therapies for inflammatory disorders involve symptomatic medications and immunosuppressive agents to control symptoms. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, fenoprofen, naproxen, tolmetin, sulindac, meclofenamate sodium, piroxicam, flurbiprofen, diclofenac, oxaprozin, nabumetone, etodolac, and ketoprofen that have analgesic and anti-inflammatory effects are used to treat inflammatory disorders. However, NSAIDs are believed not to be capable of altering progression of the disease. (Tierney *et al.* (eds.), *Current Medical Diagnosis & Treatment*, 37 ed., Appleton & Lange (1998), p793). Moreover, NSAIDs frequently cause gastrointestinal side effects, affect the lower intestinal tract causing perforation or aggravating inflammatory bowel disease, produce renal toxicity and prolong bleeding time. Corticosteroids are another class of drugs that are commonly used to control inflammatory symptoms. Corticosteroids, like NSAIDs, do not alter the natural progression of the disease, and thus, clinical manifestations of active disease commonly reappear when the drug is discontinued. The serious problem of negative reactions resulting from prolonged corticosteroid therapy (*e.g.*, osteoporosis, increased risk of infection, increased appetite, hypertension, edema, peptic ulcers, and psychoses) greatly limits its long-term use.

**[0010]** Low doses of immunosuppressive agents such as cytotoxic agents are also commonly used to in treatment of inflammatory disorders. For example, methotrexate, an antagonist of folic acid, is often used in treatment of psoriasis, rheumatoid arthritis and other inflammatory diseases. Methotrexate, like other cytotoxic agents, frequently causes stomatitis, erythema, slopecia, nausea, vomiting, diarrhea, and damage to major organs such

kidney and liver. The long-term usage of immunosuppressive agents usually leaves the patient defenseless to infections.

[0011] New therapies for inflammatory disorders are constantly being sought. In particular, any new treatment that reduces the dosage and/or frequency of administration of agents currently being used, or is capable of making a currently used treatment more effective is constantly being sought.

### 2.2.1 Asthma

[0012] About 12 million people in the U.S. have asthma and it is the leading cause of hospitalization for children. *The Merck Manual of Diagnosis and Therapy* (17th ed., 1999).

[0013] Asthma is an inflammatory disease of the lung that is characterized by airway hyperresponsiveness (“AHR”), bronchoconstriction (*i.e.*, wheezing), eosinophilic inflammation, mucus hypersecretion, subepithelial fibrosis, and elevated IgE levels. Asthmatic attacks can be triggered by environmental factors (*e.g.* acarids, insects, animals (*e.g.*, cats, dogs, rabbits, mice, rats, hamsters, guinea pigs, mice, rats, and birds), fungi, air pollutants (*e.g.*, tobacco smoke), irritant gases, fumes, vapors, aerosols, or chemicals, or pollen), exercise, or cold air. The cause(s) of asthma is unknown. However, it has been speculated that family history of asthma (London *et al.*, 2001, *Epidemiology* 12(5):577-83), early exposure to allergens, such as dust mites, tobacco smoke, and cockroaches (Melen *et al.*, 2001, 56(7):646-52), and respiratory infections (Wenzel *et al.*, 2002, *Am J Med*, 112(8):672-33 and Lin *et al.*, 2001, *J Microbiol Immuno Infect*, 34(4):259-64) may increase the risk of developing asthma.

[0014] Asthma may be identified by recurrent wheezing and intermittent air flow limitation. An asthmatic tendency may be quantified by the measurement of bronchial hyper-rsponsiveness in which an individual’s dose-response curve to a broncho-constrictor such as histamine or methacholine is constricted. The curve is commonly summarized by the dose which results in a 20% fall in air flow (PD20) or the slope of the curve between the initial air flow measurement and the last dose given (slope).

[0015] Current therapies are mainly aimed at managing asthma and include the administration of  $\beta$ -adrenergic drugs (*e.g.* epinephrine and isoproterenol), theophylline, anticholinergic drugs (*e.g.*, atropine and ipratorpium bromide), corticosteroids, and leukotriene inhibitors. These therapies are associated with side effects such as drug interactions, dry mouth, blurred vision, growth suppression in children, and osteoporosis in

menopausal women. Cromolyn and nedocromil are administered prophylactically to inhibit mediator release from inflammatory cells, reduce airway hyperresponsiveness, and block responses to allergens. However, there are no current therapies available that prevent the development of asthma in subjects at increased risk of developing asthma. Thus, new therapies with fewer side effects and better prophylactic and/or therapeutic efficacy are needed for asthma.

### **2.2.2 Allergies**

**[0016]** A common cause of inflammation are allergies. Immune-mediated allergic (hypersensitivity) reactions are classified into four types (I-IV) according to the underlying mechanisms leading to the manifestation of the allergic symptoms. Type I allergic reactions are immediate hypersensitivity reactions characterized by IgE-mediated release of vasoactive substances such as histamine from mast cells and basophils. Over hours, the mast cells and basophils release proinflammatory cytokines producing vasodilation, increased capillary permeability, glandular hypersecretion, smooth muscle spasm, and tissue infiltration with eosinophils and other inflammatory cells.

**[0017]** Type II allergic reactions are cytotoxic hypersensitivity reactions and involve IgG or IgM antibodies bound to cell surface antigens with subsequent complement fixation. Certain cytotoxic cells, such as killer T cells or macrophages, are activated, bind to cells coated with IgG and destroy the target cells. Type II reactions may result in cytolysis or tissue damage.

**[0018]** Type III reactions are immune-complex reactions resulting from deposits of circulating antigen-antibody immune complexes in blood vessels or tissues. Acute inflammation results from the immune-complex initiating a sequence of events that results in polymorphonuclear cell migration and release of lysosomal proteolytic enzymes and permeability factors in tissues.

**[0019]** Type IV reactions are delayed hypersensitivity reactions caused by sensitized T lymphocytes after contact with a specific antigen. Activated sensitized T lymphocytes cause immunologic injury by direct toxic effect or through release of lymphokines and other soluble substances. The activated T lymphocytes may also release cytokines that affect the activity of macrophages, neutrophils, and lymphoid killer cells.

**[0020]** Allergic reactions can be immediate, late-phase, or chronic. Continuous or chronic exposure to an allergen can result in chronic allergic inflammation. Tissues of sites



of chronic inflammation contain eosinophils and T cells that release mediators that can cause tissue damage, increased inflammation, and increased sensitivity.

[0021] Currently, allergic reactions are treated with drugs such as antihistamines, corticosteroids, vasodilators, bronchodilators, leukotriene inhibitors, and immunomodulators which attempt to alleviate the symptoms associated with the allergic reaction. Present therapies for allergic reactions produce negative side effects or have limited use. For example, high doses of antihistamines and corticosteroids have deleterious side effects (*e.g.*, central nervous system disturbance, constipation, *etc.*). Vasodilators pose increased risks to patients with certain conditions such as hypertension, cardiovascular disease, hyperthyroidism and may cause death from cerebrovascular hemorrhage or cardiac arrhythmia. Thus, other methods for treating allergic reactions are needed.

### 2.3 RESPIRATORY INFECTIONS

[0022] Respiratory infections are common infections of the upper respiratory tract (*e.g.*, nose, ears, sinuses, and throat) and lower respiratory tract (*e.g.*, trachea, bronchial tubes, and lungs). Symptoms of upper respiratory infection include runny or stuffy nose, irritability, restlessness, poor appetite, decreased activity level, coughing, and fever. Viral upper respiratory infections cause and/or are associated with sore throats, colds, croup, and the flu. Examples of viruses that cause upper respiratory tract infections include rhinoviruses and influenza viruses A and B. Common upper respiratory bacterial infections cause and/or are associated with, for example, whooping cough and strep throat. An example of a bacteria that causes an upper respiratory tract infection is *Streptococcus*.

[0023] Clinical manifestations of a lower respiratory infection include shallow coughing that produces sputum in the lungs, fever, and difficulty breathing. Examples of lower respiratory viral infections are parainfluenza virus infections (“PIV”), respiratory syncytial virus (“RSV”) infections, and bronchiolitis (caused by RSV, PIV, influenza virus, mycoplasma, and some adenoviruses). Examples of bacteria that cause lower respiratory tract infections include *Streptococcus pneumoniae* that causes pneumococcal pneumonia and *Mycobacterium tuberculosis* that causes tuberculosis. Respiratory infections caused by fungi include systemic candidiasis, blastomycosis, cryptococcosis, coccidioidomycosis, and aspergillosis. Respiratory infections may be primary or secondary infections.

[0024] Current therapies for respiratory infections involve the administration of anti-viral agents, anti-bacterial, and anti-fungal agents for the treatment, prevention, or amelioration of viral, bacterial, and fungal respiratory infections, respectively.

Unfortunately, in regard to certain infections, there are no therapies available, infections have been proven to be refractory to therapies, or the occurrence of side effects outweighs the benefits of the administration of a therapy to a subject. The use of anti-bacterial agents for the treatment of bacterial respiratory infections may also produce side effects or result in resistant bacterial strains. The administration of anti-fungal agents may cause renal failure or bone marrow dysfunction and may not be effective against fungal infection in patients with suppressed immune systems. Additionally, the infection causing microorganism (*e.g.*, virus, bacterium, or fungus) may be resistant or develop resistance to the administered therapeutic agent or combination of therapeutic agents. In fact, microorganisms that develop resistance to administered therapeutic agents often develop pleiotropic drug or multidrug resistance, that is, resistance to therapeutic agents that act by mechanisms different from the mechanisms of the administered agents. Thus, as a result of drug resistance, many infections prove refractory to a wide array of standard treatment protocols. Therefore, new therapies for the treatment, prevention, and amelioration of respiratory infections and symptoms thereof are needed.

### **2.3.1 Viral Respiratory Infections**

#### **2.3.1.1 Parainfluenza Virus Infections**

[0025] Parainfluenza viral (“PIV”) infection results in serious respiratory tract disease in infants and children. (Tao *et al.*, 1999, Vaccine 17: 1100-08). Infectious parainfluenza viral infections account for approximately 20% of all hospitalizations of pediatric patients suffering from respiratory tract infections worldwide. *Id.*

[0026] PIV is a member of the paramyxovirus genus of the paramyxoviridae family. PIV is made up of two structural modules: (1) an internal ribonucleoprotein core or nucleocapsid, containing the viral genome, and (2) an outer, roughly spherical lipoprotein envelope. Its genome is a single strand of negative sense RNA, approximately 15,456 nucleotides in length, encoding at least eight polypeptides. These proteins include, but are not limited to, the nucleocapsid structural protein (NP, NC, or N depending on the genera), the phosphoprotein (P), the matrix protein (M), the fusion glycoprotein (F), the hemagglutinin-neuraminidase glycoprotein (HN), the large polymerase protein (L), and the C and D proteins of unknown function. *Id.*

[0027] The parainfluenza nucleocapsid protein (NP, NC, or N) consists of two domains within each protein unit including an amino-terminal domain, comprising about

two-thirds of the molecule, which interacts directly with the RNA, and a carboxyl-terminal domain, which lies on the surface of the assembled nucleocapsid. A hinge is thought to exist at the junction of these two domains thereby imparting some flexibility to this protein (see Fields *et al.* (ed.), 1991, *Fundamental Virology*, 2nd ed., Raven Press, New York, incorporated by reference herein in its entirety). The matrix protein (M), is apparently involved with viral assembly and interacts with both the viral membrane as well as the nucleocapsid proteins. The phosphoprotein (P), which is subject to phosphorylation, is thought to play a regulatory role in transcription and may also be involved in methylation, phosphorylation and polyadenylation. The fusion glycoprotein (F) interacts with the viral membrane and is first produced as an inactive precursor then cleaved post-translationally to produce two disulfide linked polypeptides. The active F protein is also involved in penetration of the parainfluenza virion into host cells by facilitating fusion of the viral envelope with the host cell plasma membrane. *Id.* The glycoprotein, hemagglutinin-neuraminidase (HN), protrudes from the envelope allowing the virus to contain both hemagglutinin and neuraminidase activities. HN is strongly hydrophobic at its amino terminal which functions to anchor the HN protein into the lipid bilayer. *Id.* Finally, the large polymerase protein (L) plays an important role in both transcription and replication. *Id.*

[0028] Currently, therapies for PIV comprises treatment of specific symptoms. In most cases rest, fluids, and a comfortable environment are sufficient therapy for a PIV infection. In cases in which fever is high, acetaminophen is recommended over aspirin, especially in children to avoid the risk of Reye's syndrome with influenza. For croup associated with PIV infection, therapies such as humidified air, oxygen, aerosolized racemic epinephrine, and oral dexamethasone (a steroid) are recommended to decrease upper airway swelling and intravenous fluids are administered for dehydration. Therapy for bronchiolitis associated with PIV infection include supportive therapy (*e.g.*, oxygen, humidified air, chest clapping, and postural drainage to remove secretions, rest, and clear fluids) and administration of albuterol or steroids. Antibiotic, anti-viral, and/or antifungal agents may be administered to prevent secondary respiratory infections. See *Merck Manual of Diagnosis and Therapy* (17th ed., 1999).

#### **2.3.1.2 Respiratory Syncytial Virus Infections**

[0029] Respiratory syncytial virus ("RSV") is the leading cause of serious lower respiratory tract disease in infants and children (Feigen *et al.*, eds., 1987, *Textbook of*

*Pediatric Infectious Diseases*, WB Saunders, Philadelphia at pages 1653-1675; New Vaccine Development, Establishing Priorities, Vol. 1, 1985, National Academy Press, Washington DC at pages 397-409; and Ruuskanen *et al.*, 1993, *Curr. Probl. Pediatr.* 23:50-79). The yearly epidemic nature of RSV infection is evident worldwide, but the incidence and severity of RSV disease in a given season vary by region (Hall, C.B., 1993, *Contemp. Pediatr.* 10:92-110). In temperate regions of the northern hemisphere, it usually begins in late fall and ends in late spring. Primary RSV infection occurs most often in children from 6 weeks to 2 years of age and uncommonly in the first 4 weeks of life during nosocomial epidemics (Hall *et al.*, 1979, *New Engl. J. Med.* 300:393-396). Children at increased risk from RSV infection include, but are not limited to, preterm infants (Hall *et al.*, 1979, *New Engl. J. Med.* 300:393-396) and children with bronchopulmonary dysplasia (Groothuis *et al.*, 1988, *Pediatrics* 82:199-203), congenital heart disease (MacDonald *et al.*, *New Engl. J. Med.* 307:397-400), congenital or acquired immunodeficiency (Ogra *et al.*, 1988, *Pediatr. Infect. Dis. J.* 7:246-249; and Pohl *et al.*, 1992, *J. Infect. Dis.* 165:166-169), and cystic fibrosis (Abman *et al.*, 1988, *J. Pediatr.* 113:826-830). The fatality rate in infants with heart or lung disease who are hospitalized with RSV infection is 3%-4% (Navas *et al.*, 1992, *J. Pediatr.* 121:348-354).

**[0030]** RSV infects adults as well as infants and children. In healthy adults, RSV causes predominantly upper respiratory tract disease. It has recently become evident that some adults, especially the elderly, have symptomatic RSV infections more frequently than had been previously reported (Evans, A.S., eds., 1989, *Viral Infections of Humans Epidemiology and Control*, 3rd ed., Plenum Medical Book, New York at pages 525-544). Several epidemics also have been reported among nursing home patients and institutionalized young adults (Falsey, A.R., 1991, *Infect. Control Hosp. Epidemiol.* 12:602-608; and Garvie *et al.*, 1980, *Br. Med. J.* 281:1253-1254). Finally, RSV may cause serious disease in immunosuppressed persons, particularly bone marrow transplant patients (Hertz *et al.*, 1989, *Medicine* 68:269-281).

**[0031]** Therapies available for the treatment of established RSV disease are limited. Severe RSV disease of the lower respiratory tract often requires considerable supportive care, including administration of humidified oxygen and respiratory assistance (Fields *et al.*, eds, 1990, *Fields Virology*, 2nd ed., Vol. 1, Raven Press, New York at pages 1045-1072).

**[0032]** While a vaccine might prevent RSV infection, no vaccine is yet licensed for this indication. A major obstacle to vaccine development is safety. A formalin-inactivated

vaccine, though immunogenic, unexpectedly caused a higher and more severe incidence of lower respiratory tract disease due to RSV in immunized infants than in infants immunized with a similarly prepared trivalent parainfluenza vaccine (Kim *et al.*, 1969, *Am. J. Epidemiol.* 89:422-434; and Kapikian *et al.*, 1969, *Am. J. Epidemiol.* 89:405-421). Several candidate RSV vaccines have been abandoned and others are under development (Murphy *et al.*, 1994, *Virus Res.* 32:13-36), but even if safety issues are resolved, vaccine efficacy must also be improved. A number of problems remain to be solved. Immunization would be required in the immediate neonatal period since the peak incidence of lower respiratory tract disease occurs at 2-5 months of age. The immaturity of the neonatal immune response together with high titers of maternally acquired RSV antibody may be expected to reduce vaccine immunogenicity in the neonatal period (Murphy *et al.*, 1988, *J. Virol.* 62:3907-3910; and Murphy *et al.*, 1991, *Vaccine* 9:185-189). Finally, primary RSV infection and disease do not protect well against subsequent RSV disease (Henderson *et al.*, 1979, *New Engl. J. Med.* 300:530-534).

**[0033]** Currently, the only approved approach to prophylaxis of RSV disease is passive immunization. Initial evidence suggesting a protective role for IgG was obtained from observations involving maternal antibody in ferrets (Prince, G.A., Ph.D. diss., University of California, Los Angeles, 1975) and humans (Lambrecht *et al.*, 1976, *J. Infect. Dis.* 134:211-217; and Glezen *et al.*, 1981, *J. Pediatr.* 98:708-715). Hemming *et al.* (Morell *et al.*, eds., 1986, *Clinical Use of Intravenous Immunoglobulins*, Academic Press, London at pages 285-294) recognized the possible utility of RSV antibody in treatment or prevention of RSV infection during studies involving the pharmacokinetics of an intravenous immune globulin (IVIG) in newborns suspected of having neonatal sepsis. They noted that one infant, whose respiratory secretions yielded RSV, recovered rapidly after IVIG infusion. Subsequent analysis of the IVIG lot revealed an unusually high titer of RSV neutralizing antibody. This same group of investigators then examined the ability of hyperimmune serum or immune globulin, enriched for RSV neutralizing antibody, to protect cotton rats and primates against RSV infection (Prince *et al.*, 1985, *Virus Res.* 3:193-206; Prince *et al.*, 1990, *J. Virol.* 64:3091-3092; Hemming *et al.*, 1985, *J. Infect. Dis.* 152:1083-1087; Prince *et al.*, 1983, *Infect. Immun.* 42:81-87; and Prince *et al.*, 1985, *J. Virol.* 55:517-520). Results of these studies suggested that RSV neutralizing antibody given prophylactically inhibited respiratory tract replication of RSV in cotton rats. When given therapeutically, RSV antibody reduced pulmonary viral replication both in cotton rats

and in a nonhuman primate model. Furthermore, passive infusion of immune serum or immune globulin did not produce enhanced pulmonary pathology in cotton rats subsequently challenged with RSV.

**[0034]** Recent clinical studies have demonstrated the ability of this passively administered RSV hyperimmune globulin (RSV IVIG) to protect at-risk children from severe lower respiratory infection by RSV (Groothuis *et al.*, 1993, *New Engl. J. Med.* 329:1524-1530; and The PREVENT Study Group, 1997, *Pediatrics* 99:93-99). While this is a major advance in preventing RSV infection, this therapy poses certain limitations in its widespread use. First, RSV IVIG must be infused intravenously over several hours to achieve an effective dose. Second, the concentrations of active material in hyperimmune globulins are insufficient to treat adults at risk or most children with compromised cardiopulmonary function. Third, intravenous infusion necessitates monthly hospital visits during the RSV season. Finally, it may prove difficult to select sufficient donors to produce a hyperimmune globulin for RSV to meet the demand for this product. Currently, only approximately 8% of normal donors have RSV neutralizing antibody titers high enough to qualify for the production of hyperimmune globulin.

**[0035]** One way to improve the specific activity of the immunoglobulin would be to develop one or more highly potent RSV neutralizing monoclonal antibodies (MAbs). Such MAbs should be human or humanized in order to retain favorable pharmacokinetics and to avoid generating a human anti-mouse antibody response, as repeat dosing would be required throughout the RSV season. Two glycoproteins, F and G, on the surface of RSV have been shown to be targets of neutralizing antibodies (Fields *et al.*, 1990, *supra*; and Murphy *et al.*, 1994, *supra*). These two proteins are also primarily responsible for viral recognition and entry into target cells; G protein binds to a specific cellular receptor and the F protein promotes fusion of the virus with the cell. The F protein is also expressed on the surface of infected cells and is responsible for subsequent fusion with other cells leading to syncytia formation. Thus, antibodies to the F protein may directly neutralize virus or block entry of the virus into the cell or prevent syncytia formation. Although antigenic and structural differences between A and B subtypes have been described for both the G and F proteins, the more significant antigenic differences reside on the G glycoprotein, where amino acid sequences are only 53% homologous and antigenic relatedness is 5% (Walsh *et al.*, 1987, *J. Infect. Dis.* 155:1198-1204; and Johnson *et al.*, 1987, *Proc. Natl. Acad. Sci. USA* 84:5625-5629). Conversely, antibodies raised to the F protein show a high degree of

cross-reactivity among subtype A and B viruses. Comparison of biological and biochemical properties of 18 different murine MAbs directed to the RSV F protein resulted in the identification of three distinct antigenic sites that are designated A, B, and C. (Beeler and Coelingh, 1989, J. Virol. 7:2941-2950). Neutralization studies were performed against a panel of RSV strains isolated from 1956 to 1985 that demonstrated that epitopes within antigenic sites A and C are highly conserved, while the epitopes of antigenic site B are variable.

[0036] A humanized antibody directed to an epitope in the A antigenic site of the F protein of RSV, palivizumab (SYNAGIS®), is approved for intramuscular administration to pediatric patients for prevention of serious lower respiratory tract disease caused by RSV at recommended monthly doses of 15 mg/kg of body weight throughout the RSV season (November through April in the northern hemisphere). Palivizumab (SYNAGIS®) is a composite of human (95%) and murine (5%) antibody sequences. See, Johnson *et al.*, 1997, J. Infect. Diseases 176:1215-1224 and U.S. Patent No. 5,824,307, the entire contents of which are incorporated herein by reference. The human heavy chain sequence was derived from the constant domains of human IgG<sub>1</sub> and the variable framework regions of the VH genes of Cor (Press *et al.*, 1970, Biochem. J. 117:641-660) and Cess (Takashi *et al.*, 1984, Proc. Natl. Acad. Sci. USA 81:194-198). The human light chain sequence was derived from the constant domain of C<sub>κ</sub> and the variable framework regions of the VL gene K104 with J<sub>κ</sub>-4 (Bentley *et al.*, 1980, Nature 288:5194-5198). The murine sequences derived from a murine monoclonal antibody, Mab 1129 (Beeler *et al.*, 1989, J. Virology 63:2941-2950), in a process which involved the grafting of the murine complementarity determining regions into the human antibody frameworks.

### **2.3.1.3 Avian & Human Metapneumovirus**

[0037] Recently, a new member of the *Paramyxoviridae* family has been isolated from 28 children with clinical symptoms reminiscent of those caused by human respiratory syncytial virus (“hRSV”) infection, ranging from mild upper respiratory tract disease to severe bronchiolitis and pneumonia (Van Den Hoogen *et al.*, 2001, Nature Medicine 7:719-724). The new virus was named human metapneumovirus (hMPV) based on sequence homology and gene constellation. The study further showed that by the age of five years virtually all children in the Netherlands have been exposed to hMPV and that the virus has been circulating in humans for at least half a century.

[0038] The genomic organization of human metapneumovirus is described in van den Hoogen *et al.*, 2002, Virology 295:119-132. Human metapneumovirus has recently been isolated from patients in North America (Peret *et al.*, 2002, J. Infect. Diseases 185:1660-1663).

[0039] Human metapneumovirus is related to avian metapneumovirus. For example, the F protein of hMPV is highly homologous to the F protein of avian pneumovirus ("APV"). Alignment of the human metapneumoviral F protein with the F protein of an avian pneumovirus isolated from Mallard Duck shows 85.6% identity in the ectodomain. Alignment of the human metapneumoviral F protein with the F protein of an avian pneumovirus isolated from Turkey (subgroup B) shows 75% identity in the ectodomain. See, *e.g.*, co-owned and co-pending Provisional Application No. 60/358,934, entitled "Recombinant Parainfluenza Virus Expression Systems and Vaccines Comprising Heterologous Antigens Derived from Metapneumovirus," filed on February 21, 2002, by Haller and Tang, which is incorporated herein by reference in its entirety.

[0040] Respiratory disease caused by an APV was first described in South Africa in the late 1970s (Buys *et al.*, 1980, Turkey 28:36-46) where it had a devastating effect on the turkey industry. The disease in turkeys was characterized by sinusitis and rhinitis and was called turkey rhinotracheitis (TRT). The European isolates of APV have also been strongly implicated as factors in swollen head syndrome (SHS) in chickens (O'Brien, 1985, Vet. Rec. 117:619-620). Originally, the disease appeared in broiler chicken flocks infected with Newcastle disease virus (NDV) and was assumed to be a secondary problem associated with Newcastle disease (ND). Antibody against European APV was detected in affected chickens after the onset of SHS (Cook *et al.*, 1988, Avian Pathol. 17:403-410), thus implicating APV as the cause.

[0041] The avian pneumovirus is a single stranded, non-segmented RNA virus that belongs to the sub-family *Pneumovirinae* of the family *Paramyxoviridae*, genus metapneumovirus (Cavanagh and Barrett, 1988, Virus Res. 11:241-256; Ling *et al.*, 1992, J. Gen. Virol. 73:1709-1715; Yu *et al.*, 1992, J. Gen. Virol. 73:1355-1363). The *Paramyxoviridae* family is divided into two sub-families: the *Paramyxovirinae* and *Pneumovirinae*. The subfamily *Paramyxovirinae* includes, but is not limited to, the genera: Paramyxovirus, Rubulavirus, and Morbillivirus. Recently, the sub-family *Pneumovirinae* was divided into two genera based on gene order, *i.e.*, *pneumovirus* and *metapneumovirus* (Naylor *et al.*, 1998, J. Gen. Virol., 79:1393-1398; Pringle, 1998, Arch. Virol. 143:1449-



1159). The *pneumovirus* genus includes, but is not limited to, human respiratory syncytial virus (hRSV), bovine respiratory syncytial virus (bRSV), ovine respiratory syncytial virus, and mouse pneumovirus. The *metapneumovirus* genus includes, but is not limited to, European avian pneumovirus (subgroups A and B), which is distinguished from hRSV, the type species for the genus *pneumovirus* (Naylor *et al.*, 1998, J. Gen. Virol., 79:1393-1398; Pringle, 1998, Arch. Virol. 143:1449-1159). The US isolate of APV represents a third subgroup (subgroup C) within *metapneumovirus* genus because it has been found to be antigenically and genetically different from European isolates (Seal, 1998, Virus Res. 58:45-52; Senne *et al.*, 1998, In: Proc. 47th WPDC, California, pp. 67-68).

[0042] Electron microscopic examination of negatively stained APV reveals pleomorphic, sometimes spherical, virions ranging from 80 to 200 nm in diameter with long filaments ranging from 1000 to 2000 nm in length (Collins and Gough, 1988, J. Gen. Virol. 69:909-916). The envelope is made of a membrane studded with spikes 13 to 15 nm in length. The nucleocapsid is helical, 14 nm in diameter and has 7 nm pitch. The nucleocapsid diameter is smaller than that of the genera Paramyxovirus and Morbillivirus, which usually have diameters of about 18 nm.

[0043] Avian pneumovirus infection is an emerging disease in the USA despite its presence elsewhere in the world in poultry for many years. In May 1996, a highly contagious respiratory disease of turkeys appeared in Colorado, and an APV was subsequently isolated at the National Veterinary Services Laboratory (NVSL) in Ames, Iowa (Senne *et al.*, 1997, Proc. 134th Ann. Mtg., AVMA, pp. 190). Prior to this time, the United States and Canada were considered free of avian pneumovirus (Pearson *et al.*, 1993, In: Newly Emerging and Re-emerging Avian Diseases: Applied Research and Practical Applications for Diagnosis and Control, pp. 78-83; Hecker and Myers, 1993, Vet. Rec. 132:172). Early in 1997, the presence of APV was detected serologically in turkeys in Minnesota. By the time the first confirmed diagnosis was made, APV infections had already spread to many farms. The disease is associated with clinical signs in the upper respiratory tract: foamy eyes, nasal discharge and swelling of the sinuses. It is exacerbated by secondary infections. Morbidity in infected birds can be as high as 100%. The mortality can range from 1 to 90% and is highest in six to twelve week old poults.

[0044] Avian pneumovirus is transmitted by contact. Nasal discharge, movement of affected birds, contaminated water, contaminated equipment; contaminated feed trucks and load-out activities can contribute to the transmission of the virus. Recovered turkeys are

thought to be carriers. Because the virus is shown to infect the epithelium of the oviduct of laying turkeys and because APV has been detected in young poults, egg transmission is considered a possibility.

[0045] Based upon the recent work with hMPV, hMPV likewise appears to be a significant factor in human, particularly, juvenile respiratory disease.

[0046] Thus, these three viruses, RSV, hMPV, and PIV, cause a significant portion of human respiratory disease. Accordingly, a broad spectrum therapy is needed to reduce the incidence of viral respiratory disease caused by these viruses.

### **2.3.2 Bacterial Respiratory Infections**

#### **2.3.2.1 Bacterial Pneumonia**

[0047] There are about 2 million cases of pneumonia each year of which 40,000 to 70,000 result in death. The *Merck Manual of Diagnosis and Therapy* (17th ed. 1999). Although certain viruses and fungi cause pneumonia, most cases of pneumonia in adults are caused by bacteria such as *Streptococcus pneumonia*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Chlamydia pneumoniae*, *C. psittaci*, *C. trachomatis*, *Moraxella (Branhamella) catarrhalis*, *Legionella pneumophila*, *Klebsiella pneumoniae*, and other gram-negative bacilli. *Id.*

[0048] Pneumonia is usually spread by inhaling droplets small enough to reach the alveoli and aspirating secretions from the upper airways. *Id.* Alcoholics, institutionalized persons, cigarette smokers, patients with heart failure, patients with chronic obstructive airway disease, the elderly, children, infants, infants born prematurely, patients with compromised immune systems, and patients with dysphagia are at greater risk of developing pneumonia. *Id.*

[0049] Pneumonia is diagnosed based on characteristic symptoms and an infiltrate on chest x-ray. *Id.* Common symptoms of pneumonia include cough, fever, sputum production, tachypnea, and crackles with bronchial breath sounds. *Id.* Determination of the specific pathogen causing the pneumonia cannot be made in about 30-50% of patients and specimens may be misleading because of normal flora may contaminate samples through the upper airways. *Id.* Special culture techniques, special stains, serologic assays, or lung biopsies may be used for diagnosis. *Id.*

[0050] Therapies for the treatment of pneumonia consists of respiratory support, such as oxygen, and antibiotics based on determination of the specific bacteria and/or

according to the patient's age, epidemiology, host risk factors, and severity of illness. *Id.* For example, in cases of Staphylococcal pneumonia, anti-bacterial therapy comprises administration of penicillin (*e.g.*, oxacillin and nafcillin) or cephalosporin (*e.g.* cephalothin or cefamandol, cefazolin, and cefuroxime). *Id.* In cases of streptococcal pneumonia, anti-bacterial therapy comprises administration of penicillin, cephalosporins, erythromycin, or clindamycin. *Id.*

[0051] The administration of antibiotics may result in side effects, toxicity, and development of antibiotic resistant strains. In addition, because the pathogen causing pneumonia is difficult to diagnose, the use of antibiotics may be ineffective since both viruses and fungi also cause pneumonia. Thus, new therapies for pneumonia are desired.

#### **2.3.2.2 Tuberculosis**

[0052] *Mycobacterium tuberculosis* infects 1.9 billion and the active disease, tuberculosis ("TB") results in 1.9 million deaths around the world each year. (Dye *et al.*, 1999, JAMA 282:677-686). After a century of steadily declining rates of TB cases in the United States, the downward trend was reversed in the late 1980s as a result of the emergence of a multidrug-resistant strain of *M. tuberculosis*, the HIV epidemic, and the influx of immigrants. (Navin *et al.*, 2002, Emerg. Infect. Dis. 8:11).

[0053] *M. tuberculosis* is an obligate aerobe, nonmotile rod-shaped bacterium. In classic cases of tuberculosis, *M. tuberculosis* complexes are in the well-aerated upper lobes of the lungs. *M. tuberculosis* are classified as acid-fast bacteria due to the impermeability of the cell wall by certain dyes and stains. The cell wall of *M. tuberculosis*, composed of peptidoglycan and complex lipids, is responsible for the bacterium's resistance to many antibiotics, acidic and alkaline compounds, osmotic lysis, and lethal oxidations, and survival inside macrophages.

[0054] TB progresses in five stages. In the first stage, the subject inhales the droplet nuclei containing less than three bacilli. Although alveolar macrophages take up the *M. tuberculosis*, the macrophages are not activated and do not destroy the bacterium. Seven to 21 days after the initial infection, the *M. tuberculosis* multiplies within the macrophages until the macrophages burst, which attracts additional macrophages to the site of infection that phagocytose the *M. tuberculosis*, but are not activated and thus do not destroy the *M. tuberculosis*. In stage 3, lymphocytes, particularly T-cells, are activated and cytokines, including IFN activate macrophages capable of destroying *M. tuberculosis* are produced. At this stage, the patient is tuberculin-positive and a cell mediated immune response,

including activated macrophages releasing lytic enzymes and T cell secreting cytokines, is initiated. Although, some macrophages are activated against the *M. tuberculosis*, the bacteria continue to multiply within inactivated macrophages and begin to grow tubercles which are characterized by semi-solid centers. In stage 4, tubercles may invade the bronchus, other parts of the lung, and the blood supply line and the patient may exhibit secondary lesions in other parts of the body, including the genitourinary system, bones, joints, lymph nodes, and peritoneum. In the final stage, the tubercles liquify inducing increased growth of *M. tuberculosis*. The large bacterium load causes the walls of nearby bronchi to rupture and form cavities that enables the infection to spread quickly to other parts of the lung.

[0055] Current therapies available for TB comprise an initial two month regime of multiple antibiotics, such as rifampcin, isoniazid, pyranzinamide, ethambutol, or streptomycin. In the next four months, only rifampicin and isoniazid are administered to destroy persisting *M. tuberculosis*. Although proper prescription and patient compliance results in a cure in most cases, the number of deaths from TB has been on the rise as a result of the emergence of new *M. tuberculosis* strains resistant to current antibiotic therapies. (Rattan *et al.*, 1998, Emerging Infectious Diseases, 4(2):195-206). In addition, fatal and severe liver injury has been associated with treatment of latent TB with rifampcin and pyranzinamide. (CDC Morbidity and Mortality Weekly Report, 51(44):998-999).

### **2.3.3 Fungal Respiratory Infections**

[0056] The number of systemic invasive fungal infections rose sharply in the past decade due to the increase in the at-risk patient population as a result of organ transplants, oncology, human immunodeficiency virus, use of vascular catheters, and misuse of broad spectrum antibiotics. Dodds *et al.*, 2000 Pharmacotherapy 20(11): 1335-1355. Seventy percent of fungal-related deaths are caused by *Candida* species, *Aspergillus* species, and *Cryptococcus neoformans*. Yasuda, California Journal of Health-System Pharmacy, May/June 2001, pp. 4-11.

#### **2.3.3.1 Systemic Candidiasis**

[0057] 80% of all major systemic fungal infections are due to *Candida* species. *The Merk Manual of Diagnosis and Therapy*, 17th ed., 1999. Invasive candidiasis is most often caused by *Candida albicans*, *Candida troicalis*, and *Candida glabrata* in immunosuppressed patients. *Id.* Candidiasis is a defining opportunistic infection of AIDS, infecting the

esophagus, trachea, bronchi, and lungs. *Id.* In HIV-infected patients, candidiasis is usually mucocutaneous and infects the oropharynx, the esophagus, and the vagina. Ampel, April-June 1996, *Emerg. Infect. Dis.* 2(2): 109-116.

[0058] *Candida* species are commensals that colonize the normal gastrointestinal tract and skin. The Merck Manual of Diagnosis and Therapy, Berkow *et al.* (eds.), 17th ed., 1999. Thus, cultures of *Candida* from sputum, the mouth, urine, stool, vagina, or skin does not necessarily indicate an invasive, progressive infection. *Id.* In most cases, diagnosis of candidiasis requires presentation of a characteristic clinical lesion, documentation of histopathologic evidence of tissue invasion, or the exclusion of other causes. *Id.* Symptoms of systemic candidiasis infection of the respiratory tract are typically nonspecific, including dysphagia, coughing, and fever. *Id.*

[0059] All forms of candidiasis are considered serious, progressive, and potentially fatal. *Id.* Therapies for the treatment of candidiasis typically include the administration of the combination of the anti-fungal agents amphotericin B and flucytosine. *Id.* Unfortunately, acute renal failure has been associated with amphotericin B therapy. Dodds, *supra*. Fluconazole is not as effective as amphotericin B in treating certain species of *Candida*, but is useful as initial therapy in high oral or intravenous doses while species identification is pending. *The Merck Manual of Diagnosis and Therapy*, 17th ed., 1999. Fluconazole, however, has led to increasing treatment failures and anti-fungal resistance. Ampel, *supra*. Thus, there is a need for novel therapies for the treatment of systemic candidiasis.

#### 2.3.3.2 Aspergillosis

[0060] *Aspergillus* includes 132 species and 18 variants among which *Aspergillus fumigatus* is involved in 80% of Aspergillus-related diseases. Kurp *et al.*, 1999, *Medscape General Medicine* 1(3). *Aspergillus fumigatus* is the most common cause of invasive pulmonary aspergillosis that extends rapidly, causing progressive, and ultimately fatal respiratory failure. *The Merck Manual of Diagnosis and Therapy*, 17th ed., 1999. Patients undergoing long-term high-dose corticosteroid therapy, organ transplant patients, patients with hereditary disorders of neutrophil function, and patients infected with AIDS are at risk for aspergillosis.

[0061] Clinical manifestations of invasive pulmonary infection by *Aspergillus* include fever, cough, and chest pain. *Aspergillus* colonize preexisting cavity pulmonary lesions in the form of aspergilloma (fungus ball) which is composed of tangled masses

hyphae, fibrin exudate, and inflammatory cells encapsulated by fibrous tissue. *Id.* Aspergillomas usually form and enlarge in pulmonary cavities originally caused by bronchiectasis, neoplasm, TB, and other chronic pulmonary infections. *Id.* Most aspergillomas do not respond to or require systemic anti-fungal therapy. *Id.* However, invasive infections often progress rapidly and are fatal, thus aggressive therapy comprising IV amphotericin B or oral itraconazole is required. *Id.* Unfortunately, high-dose amphotericin B may cause renal failure and itraconazole is effective only in moderately severe cases. *Id.* Therefore, there is a need for new therapies for the treatment of aspergillosis.

#### **2.3.3.3 Cryptococcosis**

[0062] Cases of cryptococcosis were rare before the HIV epidemic. Ampel, *supra*. AIDS patients, patients with Hodgkin's or other lymphomas or sarcoidosis, and patients undergoing long-term corticosteroid therapy are at increased risk for cryptococcosis. *The Merk Manual of Diagnosis and Therapy*, 17th ed., 1999. In most cases, cryptococcal infections are self-limited, but AIDS-associated cryptococcal infection may be in the form of a severe, progressive pneumonia with acute dyspnea and primary lesions in the lungs. *Id.* In cases of progressive disseminated cryptococcosis affecting non-immunocompromised patients, chronic meningitis is most common without clinically evident pulmonary lesions. *Id.*

[0063] Immunocompetent patients do not always require the administration of a therapy to treat localized pulmonary cryptococcosis. However, when such patients are administered a therapy for the treatment of localized pulmonary cryptococcosis, it typically consists of the administration of amphotericin B with or without flucytosine. *Id.* AIDS patients are generally administered an initial therapy consisting of amphotericin B and flucytosine and then oral fluconazole thereafter to treat cryptococcosis. *Id.* Renal and hematologic function of all patients receiving amphotericin B with or without flucytosine must be evaluated before and during therapy since flucytosine blood levels must be monitored to limit toxicity and administration of flucytosine may not be safe for patients with preexisting renal failure or bone marrow dysfunction. *Id.* Thus, new therapies for the treatment of cryptococcosis are needed.

## **2.4 INTERLEUKIN-9**

**[0064]** Interleukin-9 (“IL-9”) plays a critical role in a number of antigen-induced responses in mice, such as bronchial hyperresponsiveness, epithelial mucin production, eosinophilia, elevated T cells, B cells, mast cells, macrophages, neutrophils, eosinophils, and other inflammatory cell counts in the bronchial lavage, histologic changes in the lung associated with inflammation, and elevated serum total IgE. See Levitt *et al.*, U.S. patent No. 6,261,559, herein incorporated by reference. The structural similarity observed for human and murine IL-9 genes suggests that human IL-9 has a significant role in facilitating asthmatic immune responses. IL-9 is expressed by activated T cells and mast cells and functions as a T cell growth factor, mediates the growth of erythroid progenitors, B cells, mast cells, eosinophils, and fetal thymocytes, acts synergistically with interleukin-3 (“IL-3”) to induce mast cell activation and proliferation, and promotes the production of mucin by lung epithelium. The administration of murine antibodies to human subject is associated with many drawbacks. Thus, antibodies having a low immunogenicity and a high affinity for human IL-9 would be useful to treat human patients suffering from diseases associated with IL-9 expression and/or activity such as asthma.

**[0065]** Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

## **3. SUMMARY OF THE INVENTION**

**[0066]** The present invention provides antibodies that immunospecifically bind to an interleukin-9 (“IL-9”) polypeptide (preferably, a human IL-9 polypeptide). In particular, the invention provides the following antibodies that immunospecifically bind to an IL-9 polypeptide: 4D4 or an antigen-binding fragment thereof, 4D4 H2-1 D11 or an antigen-binding fragment thereof, 4D4com-XF-9 or an antigen-binding fragment thereof, 4D4com-2F9 or an antigen-binding fragment thereof, 7F3 or an antigen-binding fragment thereof, 71A10 or an antigen-binding fragment thereof, 22D3 or an antigen-binding fragment thereof, 7F3com-2H2 or an antigen-binding fragment thereof, 7F3com-3H5 or an antigen-binding fragment thereof, and 7F3com-3D4 or an antigen-binding fragment thereof.

**[0067]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a variable heavy (“VH”) domain having an amino acid sequence of the VH domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. The present

invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a variable light (“VL”) domain having an amino acid sequence of the VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. The present invention also provides for antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VH domain and VL domain having the amino acid sequence of the VH and VL domains of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. The invention further provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising one or more VH complementarity determining regions (“CDRs”) and/or one or more VL CDRs having the amino acid sequence of one or more of the VH CDRs of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4 and/or the amino acid sequence of one or more of the VL CDRs of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4, respectively.

**[0068]** In a preferred embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, wherein the antibody comprises the VH domain and/or VL domain of 7F3com-2H2. In another preferred embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, wherein the antibody comprises one, two, or three VH CDRs (preferably including, VH CDR3) of 7F3com-2H2. In another preferred embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, wherein the antibody comprises one, two or three VL CDRs (preferably including, VL CDR3) of 7F3com-2H2. In yet another preferred embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, wherein the antibody comprises one, two or three VH CDRs and one, two or three VL CDRs of 7F3com-2H2.

**[0069]** The present invention provides for mixtures of antibodies that immunospecifically bind to an IL-9 polypeptide, wherein the mixture comprises at least one, two, three, four, five or more different antibodies of the invention. The present invention also provides for panels of antibodies that immunospecifically bind to an IL-9 polypeptide, wherein the panel has at least one, two, three, four, five or more different antibodies of the invention. In particular, the invention provides for panels of different antibodies that immunospecifically bind an IL-9 polypeptide in the milieu (*i.e.*, not bound to



the IL-9R or a subunit thereof), the receptor-bound form of an IL-9 polypeptide, and/or both the receptor-bound form of an IL-9 polypeptide and an IL-9 polypeptide in the milieu. In specific embodiments, the invention provides for panels of antibodies that have different affinities for an IL-9 polypeptide, different specificities for an IL-9 polypeptide, or different dissociation rates. The invention provides panels of at least 10, preferably at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, or at least 1000, antibodies. Panels of antibodies can be used, for example, in 96 well plates for assays such as ELISAs.

**[0070]** In a preferred embodiment, the antibodies of the invention are human or humanized antibodies. In another embodiment, the antibodies of the invention are conjugated to a detectable substance or therapeutic agent. In an alternative embodiment, the antibodies of the invention are not conjugated to a detectable substance or a therapeutic agent.

**[0071]** The present invention encompasses treatment protocols that provide better prophylactic or therapeutic profiles than current single agent therapies or combination therapies for diseases or disorders associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders associated with or characterized by aberrant expression and/or activity of the IL-9 receptor (“IL-9R”) or one or more subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, or infections (preferably, respiratory infections), or one or more symptoms thereof. In particular, the invention provides prophylactic and therapeutic protocols for the prevention, treatment, management, and/or amelioration of diseases or disorders characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, or infections (preferably, respiratory infections), or one or more symptoms thereof, comprising administering to a subject an effective amount of one or more of the antibodies of the invention. The invention also provides prophylactic and therapeutic protocols for the prevention, treatment, management, and/or amelioration of diseases or disorders characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders characterized by aberrant expression and/or activity of the IL-9 receptor or one or

more subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, or infections (preferably, respiratory infections), or one or more symptoms thereof, comprising administering to a subject an effective amount of one or more antibodies of the invention and an effective amount of at least one therapy (*e.g.*, a prophylactic or therapeutic agent) other than an antibody of the invention.

**[0072]** In one embodiment, the present invention provides methods of preventing, treating, managing, and/or ameliorating an autoimmune disorder or one or more symptoms thereof, said method comprising administering an effective amount of one or more IL-9 antibodies of the invention alone or in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, used or known to be effective in preventing, treating, managing, and/or ameliorating autoimmune diseases. In a preferred embodiment, the autoimmune disorder is rheumatoid arthritis or multiple sclerosis. Non-limiting examples of the prophylactic or therapeutic agents which can be used to prevent, treat, manage, and/or ameliorate an autoimmune disorder include anti-viral agents, an anti-bacterial agents, TNF- $\alpha$  antagonists, immunomodulatory agents, and anti-inflammatory agents. In a preferred embodiment, an effective amount of one or more antibodies of the present invention is administered in combination with an effective amount of VITAXIN™, sipilizumab (MEDI-507; MedImmune, Inc.), one or more anti-EphA2 antibodies (*see* U.S. Patent Publication No. US2004/0028685 A1, dated February 12, 2004 and U.S. Pat. Appn. No. 10/436,783, filed May 12, 2003, which are both incorporated by reference herein in their entireties), or any combination thereof to prevent, manage, treat, and/or ameliorate an autoimmune disorder or one or more symptoms thereof.

**[0073]** In one embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof, said method comprising administering an effective amount of one or more IL-9 antibodies of the invention alone or in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, used or known to be effective in preventing, treating, managing, and/or ameliorating inflammatory disorders. In a specific embodiment, the inflammatory disorder is asthma, an allergy, arthritis, or a disorder characterized by type-2 mediated inflammation. Non-limiting examples of therapies (*e.g.*, prophylactic or therapeutic agents) for the prevention, treatment, management, and/or amelioration of an inflammatory disorder

include anti-viral agents, anti-bacterial agents, anti-fungal agents, TNF- $\alpha$  antagonists, immunomodulatory agents, mast cell modulators, and anti-inflammatory agents. In a preferred embodiment, an effective amount of one or more antibodies of the present invention is administered in combination with an effective amount of VITAXIN™, siplizumab, one or more anti-EphA2 antibodies, or any combination thereof to prevent, manage, treat, and/or ameliorate an inflammatory disorder or one or more symptoms thereof.

**[0074]** In one embodiment, the present invention provides a method of preventing, treating, managing, and/or ameliorating a proliferative disorder or one or more symptoms thereof, said method comprising administering an effective amount of one or more IL-9 antibodies of the invention alone or in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, used or known to be effective in preventing, treating, managing, and/or ameliorating proliferative disorders. In a specific embodiment, the proliferative disorder is cancer, chronic obstructive pulmonary disease (“COPD”), or lung fibrosis. Non-limiting examples of the therapies (*e.g.*, prophylactic or therapeutic agents) for the prevention, treatment, management, and/or amelioration of a proliferative disorder include anti-viral agents, anti-bacterial agents, anti-fungal agents, anti-angiogenic agents, TNF- $\alpha$  antagonists, immunomodulatory agents, anti-cancer agents, and anti-inflammatory agents. In a preferred embodiment, an effective amount of one or more antibodies of the present invention is administered in combination with an effective amount of VITAXIN™, siplizumab, one or more anti-EphA2 antibodies, or any combination thereof to prevent, manage, treat, and/or ameliorate a proliferative disorder or one or more symptoms thereof.

**[0075]** In one embodiment, the present invention provides a method of preventing, treating, managing, and/or ameliorating infections, preferably respiratory infections, or one or more symptoms thereof, said method comprising administering an effective amount of one or more IL-9 antibodies of the invention alone or in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, used or known to be effective in preventing, treating, managing, and/or ameliorating infections. In certain embodiments, the infection is a pulmonary or respiratory infection. In a specific embodiment, the infection is a respiratory infection caused by a virus, bacteria, or fungus. In a more specific embodiment, the respiratory infection is caused by respiratory syncytial virus (“RSV”), parainfluenza virus

("PIV"), or human metapneumovirus ("hMPV"). Non-limiting examples of therapies (*e.g.*, prophylactic or therapeutic agents) for the prevention, treatment, management, and/or amelioration of infections include anti-viral agents, anti-bacterial agents, anti-fungal agents, immunomodulatory agents, anti-cancer agents, and anti-inflammatory agents. In a preferred embodiment, an effective amount of one or more antibodies of the present invention is administered in combination with an effective amount of VITAXIN™, siplizumab, palivizumab, NUMAX™ (MedImmune, Inc.) one or more anti-EphA2 antibodies, or any combination thereof to prevent, manage, treat, and/or ameliorate infections or one or more symptoms thereof. Examples of antibody derivatives include MT103, part of a class of antibody derivatives known as Bi-Specific T Cell Engagers (BiTE™; MedImmune, Inc.), that may also be used in combination with one or more antibodies of the present invention.

**[0076]** The present invention provides a method of diagnosing, prognosing, or monitoring a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) comprising assaying the level of IL-9 in cells or a tissue sample of a subject using an IL-9 antibody of the invention and comparing the assayed level of IL-9 with a control level (*e.g.*, PBS). An increase or decrease in the assayed level of IL-9 compared to the control level of IL-9 is indicative of a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection. The invention also provides for pharmaceutical compositions, kits, and articles of manufacture comprising one or more antibodies that immunospecifically bind to an IL-9 polypeptide with or without one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, for use in the prevention, treatment, management, and/or amelioration of a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory

infection), or one or more symptoms thereof. The kits or articles of manufacture may further comprise instructions.

### **3.1 TERMINOLOGY**

**[0077]** As used herein the term “aberrant” refers to a deviation from the norm, *e.g.*, the average healthy subject and/or a population of average healthy subjects. The term “aberrant expression,” as used herein, refers to abnormal expression of a gene product (*e.g.*, RNA, protein, polypeptide, or peptide) by a cell or subject relative to a normal, healthy cell or subject and/or a population of normal, healthy cells or subjects. Such aberrant expression may be the result of the amplification of the gene. In a specific embodiment, the term “aberrant expression” refers to abnormal expression of IL-9 and/or an IL-9R or subunit thereof by a cell or subject relative to the expression of the gene product by a normal, healthy cell or subject and/or a population of normal, healthy cells or subjects and encompasses the expression of an IL-9 and/or an IL-9R or subunit thereof gene product at an unusual location within the cell or subject, the expression of an IL-9 and/or an IL-9R or subunit thereof gene product at an altered level in the cell or subject, the expression of a mutated IL-9 and/or IL-9R or subunit thereof gene product, or a combination thereof. The term “aberrant activity”, as used herein, refers to an altered level of a gene product, the increase of an activity by a gene product, or the loss of an activity of a gene product in a cell or subject relative to a normal, healthy cell or subject and/or a population of normal, healthy cells or subjects. In specific embodiments, the term “aberrant activity” refers to an IL-9 and/or IL-9R or subunit thereof activity that deviates from that normally found in a healthy cell or subject and/or a population of normal, healthy cells or subjects (*e.g.*, an increase in IL-9’s ability to bind to its receptor). Examples of IL-9 activities include, but are not limited to, the phosphorylation of the IL-9R, the activation of Jak3, the activation of MEK, the activation of Stat 1, and the activation of Stat 3.

**[0078]** As used herein, the term “analog” in the context of a proteinaceous agent (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that possesses a similar or identical functions as a second proteinaceous agent but does not necessarily comprise a similar or identical amino acid sequence of the second proteinaceous agent, or possess a similar or identical structure of the second proteinaceous agent. A proteinaceous agent that has a similar amino acid sequence refers to a second proteinaceous agent that satisfies at least one of the following: (a) a proteinaceous agent having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at

least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a second proteinaceous agent; (b) a proteinaceous agent encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a second proteinaceous agent of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues; and (c) a proteinaceous agent encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a second proteinaceous agent. A proteinaceous agent with similar structure to a second proteinaceous agent refers to a proteinaceous agent that has a similar secondary, tertiary or quaternary structure to the second proteinaceous agent. The structure of a proteinaceous agent can be determined by methods known to those skilled in the art, including but not limited to, peptide sequencing, X-ray crystallography, nuclear magnetic resonance, circular dichroism, and crystallographic electron microscopy.

**[0079]** To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

**[0080]** The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score=50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, the NCBI website). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

**[0081]** The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

**[0082]** As used herein, the term “analog” in the context of a non-proteinaceous analog refers to a second organic or inorganic molecule which possesses a similar or identical function as a first organic or inorganic molecule and is structurally similar to the first organic or inorganic molecule.

**[0083]** As used herein, the terms “antagonist” and “antagonists” refer to any protein, polypeptide, peptide, peptidomimetic, glycoprotein, antibody, antibody fragment, carbohydrate, nucleic acid, organic molecule, inorganic molecule, large molecule, or small

molecule that blocks, inhibits, reduces or neutralizes the function, activity and/or expression of another molecule. In various embodiments, an antagonist reduces the function, activity and/or expression of another molecule by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% relative to a control such as phosphate buffered saline (PBS).

**[0084]** As used herein, the terms “antibody” and “antibodies” refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, camelised antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, single domain antibodies, Fab fragments, F(ab’) fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), intrabodies, and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site.

Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub> and IgA<sub>2</sub>) or subclass.

**[0085]** As used herein, the terms “anti-IL 9 antibodies,” “IL-9 antibodies,” “antibodies of the invention,” “antibodies of the present invention” and analogous terms refer to the antibodies described in section 5.1.

**[0086]** As used herein, the term “control IgG antibody” refers to an IgG antibody or other “control antibody” that does not immunospecifically bind to an IL-9 polypeptide and preferably does not cross-react with an IL-9 polypeptide.

**[0087]** As used herein, the term “cytokine receptor modulator” refers to an agent which modulates the phosphorylation of a cytokine receptor, the activation of a signal transduction pathway associated with a cytokine receptor, and/or the expression of a particular protein such as a cytokine or cytokine receptor. Such an agent may directly or indirectly modulate the phosphorylation of a cytokine receptor, the activation of a signal transduction pathway associated with a cytokine receptor, and/or the expression of a particular protein such as a cytokine. Thus, examples of cytokine receptor modulators include, but are not limited to, cytokines, fragments of cytokines, fusion proteins, and antibodies that immunospecifically binds to a cytokine receptor or a fragment thereof. Further, examples of cytokine receptor modulators include, but are not limited to, peptides,



polypeptides (*e.g.*, soluble cytokine receptors), fusion proteins and antibodies that immunospecifically binds to a cytokine or a fragment thereof.

**[0088]** As used herein, the term “derivative” in the context of proteinaceous agent (*e.g.*, proteins, polypeptides, peptides, and antibodies) refers to a proteinaceous agent that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions, and/or additions. The term “derivative” as used herein also refers to a proteinaceous agent which has been modified, *i.e.*, by the covalent attachment of any type of molecule to the proteinaceous agent. For example, but not by way of limitation, an antibody may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a proteinaceous agent may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a proteinaceous agent may contain one or more non-classical amino acids. A derivative of a proteinaceous agent possesses a similar or identical function as the proteinaceous agent from which it was derived.

**[0089]** As used herein, the term “derivative” in the context of a non-proteinaceous derivative refers to a second organic or inorganic molecule that is formed based upon the structure of a first organic or inorganic molecule. A derivative of an organic molecule includes, but is not limited to, a molecule modified, *e.g.*, by the addition or deletion of a hydroxyl, methyl, ethyl, carboxyl, nitril, or amine group. An organic molecule may also be esterified, alkylated and/or phosphorylated.

**[0090]** As used herein, the terms “disorder” and “disease” are used interchangeably to refer to a condition in a subject. In particular, the term “autoimmune disease” is used interchangeably with the term “autoimmune disorder” to refer to a condition in a subject characterized by cellular, tissue and/or organ injury caused by an immunologic reaction of the subject to its own cells, tissues and/or organs. The term “inflammatory disease” is used interchangeably with the term “inflammatory disorder” to refer to a condition in a subject characterized by inflammation, preferably chronic inflammation. Autoimmune disorders may or may not be associated with inflammation. Moreover, inflammation may or may not be caused by an autoimmune disorder. Certain conditions may be characterized as more

than one disorder. For example, certain conditions may be characterized as both autoimmune and inflammatory disorders.

**[0091]** As used herein, the term “effective amount” refers to the amount of a therapy (*e.g.*, a prophylactic or therapeutic agent) which is sufficient to reduce and/or ameliorate the severity and/or duration of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof), prevent the advancement of said disease or disorder, cause regression of said disease or disorder, prevent the recurrence, development, or onset of one or more symptoms associated with said disease or disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (*e.g.*, prophylactic or therapeutic agent).

**[0092]** As used herein, the term “epitopes” refers to fragments of a polypeptide or protein having antigenic or immunogenic activity in an animal, preferably in a mammal, and most preferably in a human. An epitope having immunogenic activity is a fragment of a polypeptide or protein that elicits an antibody response in an animal. An epitope having antigenic activity is a fragment of a polypeptide or protein to which an antibody immunospecifically binds as determined by any method well-known to one of skill in the art, for example by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

**[0093]** As used herein, the term “fragment” refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of a second, different polypeptide or protein. In another embodiment, a fragment of a protein or polypeptide retains at least one function of the protein or polypeptide. In

another embodiment, a fragment of a polypeptide or protein retains at least two, three, four, or five functions of the polypeptide or protein. Preferably, a fragment of an antibody that immunospecifically binds to an IL-9 polypeptide retains the ability to immunospecifically bind to an IL-9 polypeptide.

**[0094]** As used herein, the term “fusion protein” refers to a polypeptide or protein that comprises an amino acid sequence of a first polypeptide or protein or fragment, analog or derivative thereof, and an amino acid sequence of a heterologous polypeptide or protein (*i.e.*, a second polypeptide or protein or fragment, analog or derivative thereof different than the first polypeptide or protein or fragment, analog or derivative thereof). In one embodiment, a fusion protein comprises a prophylactic or therapeutic agent fused to a heterologous protein, polypeptide or peptide. In accordance with this embodiment, the heterologous protein, polypeptide or peptide may or may not be a different type of prophylactic or therapeutic agent. For example, two different proteins, polypeptides, or peptides with immunomodulatory activity may be fused together to form a fusion protein. In a preferred embodiment, fusion proteins retain or have improved activity relative to the activity of the original polypeptide or protein prior to being fused to a heterologous protein, polypeptide, or peptide.

**[0095]** As used herein, the term “host cell” includes a particular subject cell transfected or transformed with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

**[0096]** As used herein, the term “human adult” or “adult” refers to a human 18 years of age or older.

**[0097]** As used herein, the terms “human child” or “child” or variations thereof refer to a human between 24 months of age and 18 years of age.

**[0098]** As used herein, the terms “elderly human,” “elderly,” or variations thereof refer to a human 65 years old or older, preferably 70 years old or older.

**[0099]** As used herein, the terms “human infant” or “infant” or variations thereof refer to a human less than 24 months of age, preferably less than 12 months, less than 6 months, less than 3 months, less than 2 months, or less than 1 month of age.

**[00100]** As used herein, the terms “human infant born prematurely,” “preterm infant,” or “premature infant,” or variations thereof refer to a human born at less than 40 weeks of gestational age, preferably less than 35 weeks gestational age, who is less than 6 months old, preferably less than 3 months old, more preferably less than 2 months old, and most preferably less than 1 month old.

**[00101]** As used herein, the term “hybridizes under stringent conditions” describes conditions for hybridization and washing under which nucleotide sequences at least 30% (preferably, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

**[00102]** Generally, stringent conditions are selected to be about 5 to 10°C lower than the thermal melting point (T<sub>m</sub>) for the specific sequence at a defined ionic strength pH. The T<sub>m</sub> is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T<sub>m</sub>, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (for example, 10 to 50 nucleotides) and at least 60°C for long probes (for example, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents, for example, formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization.

**[00103]** In one, non-limiting example stringent hybridization conditions are hybridization at 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.1XSSC, 0.2% SDS at about 68 °C. In a preferred, non-limiting example stringent hybridization conditions are hybridization in 6XSSC at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C (*i.e.*, one or more washes at 50°C, 55°C, 60°C or 65°C). It is understood that the nucleic acids of the invention do not include nucleic acid molecules that hybridize under these conditions solely to a nucleotide sequence consisting of only A or T nucleotides.

**[00104]** As used herein, the term “IL-9 polypeptide” refers to IL-9, an analog, derivative or a fragment thereof, including mature and immature forms of IL-9 (*see*, Van Snick *et al.*, 1989, J Exp. Med. 169:363-68 and Yang *et al.*, 1989, Blood 74:1880-84, which are both incorporated by reference herein in their entireties), or a fusion protein comprising IL-9, an analog, derivative or a fragment thereof. The IL-9 polypeptide may be from any species. The nucleotide and/or amino acid sequences of IL-9 polypeptides can be found in the literature or public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing techniques known to one of skill in the art. For example, the nucleotide sequence of human IL-9 can be found in the GenBank database (*see, e.g.*, Accession No. NM\_000590; FIG. 12). The amino acid sequence of human IL-9 can be found in the GenBank database (*see, e.g.*, Accession Nos. A60480, NP\_000584 and AAC17735; FIG. 13) and in U.S. Provisional Application No. 60/371,683, entitled, “Recombinant Anti-Interleukin-9 Antibodies,” filed April 12, 2002 (the amino acid sequence of human IL-9 on page 15 is specifically incorporated herein by reference). In a preferred embodiment, an IL-9 polypeptide is human IL-9, an analog, derivative or a fragment thereof.

**[00105]** As used herein, the terms “IL-9 receptor” and “IL-9R” refer to an IL-9 receptor or an analog, derivative, or fragment thereof, or a fusion protein comprising an IL-9 receptor, an analog, derivative, or a fragment thereof. As used herein, the terms “one or more subunits” and “a subunit” in the context of an IL-9R refer to the IL-9R ligand-specific alpha subunit (“IL-9R $\alpha$ ”) and/or common  $\gamma_c$  chain (also present in IL-2R, IL-4R, IL-7R, and IL-15R complexes) of the functional IL-9R or an analog, derivative, or fragment thereof. In a preferred embodiment, a functional IL-9R mediates a proliferative response in T cells treated with IL-9 as determined by any cell proliferation assay known to those skilled in the art (*e.g.*, a [ $^3$ H]-thymidine incorporation assay or a hexosaminidase assay) (*see, e.g.*, Renauld *et al.*, 1992, Proc. Natl. Acad. Sci. USA, 89:5690-94 and Bauer *et al.*, 1998, J Biol. Chem. 273:9255-60, which are both incorporated by reference herein in their entireties). Preferably, treating a T cell line expressing a functional IL-9R (*e.g.*, TS1 RA3 cells (R&D Systems) expressing both human and murine IL-9R $\alpha$ ) with IL-9, results in a dose-dependent increase in T cell proliferation, as measured by any cell proliferation assay known to those skilled in the art (*see*, Renauld *et al.*, 1992, Proc. Natl. Acad. Sci. USA, 89:5690-94 and Bauer *et al.*, 1998, J Biol. Chem. 273:9255-60). In another preferred embodiment, a functional IL-9R, comprising the  $\gamma_c$  and IL-9R $\alpha$  chains, initiates a signaling

cascade through the Janus kinases JAK1 and JAK3, thereby activating homo- and heterodimers of the signal transducer and activator transcription (STAT) factors STAT-1, STAT-3 and STAT-5 (*see*, Bauer *et al.*, 1998, J Biol. Chem. 273:9255-60). In another preferred embodiment, a functional IL-9R may prevent apoptosis in a mechanism involving STAT-3 and STAT-5, as determined by apoptosis assays known to those skilled in the art (*see*, Bauer *et al.*, 1998, J Biol. Chem. 273:9255-60). The IL-9R or one or more subunits thereof may be from any species. The nucleotide and/or amino acid sequences of the IL-9R and the subunits thereof can be found in the literature or in public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing techniques known to one of skill in the art. For example, the nucleotide sequence of human IL-9R can be found in the GenBank database (*see, e.g.*, Accession Nos. NM\_002186, NM\_176786, and NM\_000206; FIG. 14). The amino acid sequence of human IL-9R can be found in the GenBank database (*see, e.g.*, Accession Nos. NP\_002177; NP\_789743, and NP\_000197; FIG 15) and in U.S. Provisional Application No. 60/371,683, entitled, "Recombinant Anti-Interleukin-9 Antibodies," filed April 12, 2002 (the amino acid sequence of human IL-9R on page 16 is herein specifically incorporated by reference). In a preferred embodiment, an IL-9R or one or more subunits thereof is a human IL-9R or one or more subunits thereof, an analog, derivative, or a fragment thereof.

**[00106]** As used herein, the term "immunomodulatory agent" and variations thereof including, but not limited to, immunomodulatory agents, immunomodulants or immunomodulatory drugs, refer to an agent that modulates a host's immune system. In a specific embodiment, an immunomodulatory agent is an agent that shifts one aspect of a subject's immune response. In certain embodiments, an immunomodulatory agent is an agent that inhibits or reduces a subject's immune system (*i.e.*, an immunosuppressant agent). In certain other embodiments, an immunomodulatory agent is an agent that activates or increases a subject's immune system (*i.e.*, an immunostimulatory agent). In accordance with the invention, an immunomodulatory agent used in the combination therapies of the invention does not include an antibody of the invention.

Immunomodulatory agents include, but are not limited to, small molecules, peptides, polypeptides, proteins, nucleic acids (*e.g.*, DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides or peptides), antibodies, synthetic or natural inorganic molecules, mimetic agents, and synthetic or natural organic molecules.

**[00107]** As used herein, the term “immunospecifically binds to an antigen” and analogous terms refer to peptides, polypeptides, proteins, fusion proteins and antibodies or fragments thereof that specifically bind to an antigen or a fragment and do not specifically bind to other antigens. A peptide, polypeptide, protein, or antibody that immunospecifically binds to an antigen may bind to other peptides, polypeptides, or proteins with lower affinity as determined by, *e.g.*, immunoassays, BIAcore, or other assays known in the art. Antibodies or fragments that immunospecifically bind to an antigen may be cross-reactive with related antigens. Preferably, antibodies or fragments that immunospecifically bind to an antigen do not cross-react with other antigens. An antibody binds specifically to an antigen when it binds to the antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). See, *e.g.*, Paul, ed., 1989, *Fundamental Immunology*, 2nd ed., Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity.

**[00108]** As used herein, the term “immunospecifically binds to an IL-9 polypeptide” and analogous terms refer to peptides, polypeptides, proteins, fusion proteins, and antibodies or fragments thereof that specifically bind to an IL-9 polypeptide and do not specifically bind to other polypeptides. The term “immunospecifically binds to an IL-9R” and analogous terms refer to peptides, polypeptides, proteins, fusion proteins, and antibodies or fragments thereof that specifically bind to an IL-9 receptor or one or more of subunits thereof and do not specifically bind to other receptors. A peptide, polypeptide, protein, or antibody that immunospecifically binds to an IL-9 polypeptide or an IL-9R may bind to other peptides, polypeptides, or proteins with lower affinity as determined by, *e.g.*, immunoassays, BIAcore, or other assays known in the art. Antibodies or fragments that immunospecifically bind to an IL-9 polypeptide or an IL-9R may be cross-reactive with related antigens. Preferably, antibodies or fragments that immunospecifically bind to an IL-9 polypeptide or an IL-9R thereof do not cross-react with other antigens. Antibodies or fragments that immunospecifically bind to an IL-9 polypeptide or an IL-9R can be identified, for example, by immunoassays, BIAcore, or other techniques known to those of skill in the art. An antibody or fragment thereof binds specifically to an IL-9 polypeptide or an IL-9R when it binds to an IL-9 polypeptide or IL-9R with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). See, *e.g.*,

Paul, ed., 1989, Fundamental Immunology, 2nd ed., Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity. In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide does not bind or cross-react with other antigens. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide that is a fusion protein specifically binds to the portion of the fusion protein that is IL-9.

**[00109]** As used herein, the term “in combination” refers to the use of more than one therapy (*e.g.*, more than one prophylactic agent and/or therapeutic agent). The use of the term “in combination” does not restrict the order in which therapies (*e.g.*, prophylactic and/or therapeutic agents) are administered to a subject with a disease or disorder. A first therapy (*e.g.*, a first prophylactic or therapeutic agent) can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy (*e.g.*, a second prophylactic or therapeutic agent) to a subject with a disorder.

**[00110]** As used herein, the term “isolated” in the context of an organic or inorganic molecule (whether it be a small or large molecule), other than a proteinaceous agent or nucleic acid molecule, refers to an organic or inorganic molecule substantially free of a different organic or inorganic molecule. Preferably, an organic or inorganic molecule is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% free of a second, different organic or inorganic molecule. In a preferred embodiment, an organic and/or inorganic molecule is isolated.

**[00111]** As used herein, the term “isolated” in the context of a proteinaceous agent (*e.g.*, a peptide, polypeptide, fusion protein, or antibody) refers to a proteinaceous agent which is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language “substantially free of cellular material” includes preparations of a proteinaceous agent in which the proteinaceous agent is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a proteinaceous agent that is substantially free of cellular material includes



preparations of a proteinaceous agent having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein, polypeptide, peptide, or antibody (also referred to as a "contaminating protein"). When the proteinaceous agent is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the proteinaceous agent preparation. When the proteinaceous agent is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the proteinaceous agent. Accordingly, such preparations of a proteinaceous agent have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the proteinaceous agent of interest. In a specific embodiment, proteinaceous agents disclosed herein are isolated. In a preferred embodiment, an antibody of the invention is isolated. In a specific embodiment, an "isolated" antibody is purified by a multi-step purification process that comprises three chromatography steps (cation exchange, protein A and anion exchange), a nanofiltration step, and a low pH treatment step (for a detailed description, *see* Section 6, *infra*).

**[00112]** As used herein, the term "isolated" in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized; however, "isolated" excludes members of a library of clones such as a cDNA library. In a preferred embodiment, a nucleic acid molecule encoding an antibody of the invention is isolated.

**[00113]** As used herein, the terms "manage," "managing," and "management" refer to the beneficial effects that a subject derives from a therapy (*e.g.*, a prophylactic or therapeutic agent), which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) to "manage" a disease so as to prevent the progression or worsening of the disease.

**[00114]** As used herein, the term "mast cell modulator" refers to an agent which modulates the activation of a mast cell, mast cell degranulation, and/or expression of a particular protein such as a cytokine. Such an agent may directly or indirectly modulate the

activation of a mast cell, degranulation of the mast cell, and/or the expression of a particular protein such as a cytokine. Non-limiting examples of mast cell modulators include, but are not limited to, small molecules, peptides, polypeptides, proteins, nucleic acids (*e.g.*, DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides, or peptides), fusion proteins, antibodies, synthetic or natural inorganic molecules, synthetic or natural organic molecule, or mimetic agents which inhibit and/or reduce the expression, function, and/or activity of a stem cell factor, a mast cell protease, a cytokine (such as IL-3, IL-4, and IL-9), a cytokine receptor (such as IL-3R, IL-4R, and IL-9R), and a stem cell receptor. Other non-limiting examples of mast cell modulators include, but are not limited to small molecules, peptides, polypeptides, proteins, nucleic acids (*e.g.*, DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides, or peptides), fusion proteins, antibodies, synthetic or natural inorganic molecules, synthetic or natural organic molecule, or mimetic agents which inhibit and/or reduce the expression, function and/or activity of IgE. In certain embodiments, a mast cell modulator is an agent that prevents or reduces the activation of additional mast cells following degranulation of mast cells. In other embodiments, a mast cell modulator is an agent that inhibits or reduces mast cell degranulation. In accordance with the invention, a mast cell modulator used in the combination therapies of the invention does not include an antibody of the invention.

**[00115]** As used herein, the terms “non-responsive” and refractory” describe patients treated with a currently available therapy (*e.g.*, prophylactic or therapeutic agent) for a disorder, (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof) which is not clinically adequate to relieve one or more symptoms associated with the disorder. Typically, such patients suffer from severe, persistently active disease and require additional therapy to ameliorate the symptoms associated with the disorder.

**[00116]** As used herein, the phrase “pharmaceutically acceptable” means approved by a regulatory agency of the federal or a state government, or listed in the U.S.

Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia for use in animals, and more particularly, in humans.

**[00117]** As used herein, the terms “prevent,” “preventing,” and “prevention” refer to the inhibition of the development or onset of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof) or the prevention of the recurrence, onset, or development of one or more symptoms of such a disease or disorder in a subject resulting from the administration of a therapy (*e.g.*, a prophylactic or therapeutic agent), or the administration of a combination of therapies (*e.g.*, a combination of prophylactic and/or therapeutic agents).

**[00118]** As used herein, the terms “prophylactic agent” and “prophylactic agents” refer to any agent(s) which can be used in the prevention of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In certain embodiments, the term “prophylactic agent” refers to an antibody that immunospecifically binds to an IL-9 polypeptide. In certain other embodiments, the term “prophylactic agent” refers to an agent other than an antibody that immunospecifically binds to an IL-9 polypeptide. Preferably, a prophylactic agent is an agent which is known to be useful to or has been or is currently being used to prevent or impede the onset, development, progression and/or severity of such a disease or disorder. Prophylactic agents may be characterized as different agents based upon one or more effects that the agents have *in vitro* and/or *in vivo*. For example, a mast cell modulator may also be characterized as an immunomodulatory agent.

**[00119]** As used herein, the term “prophylactically effective amount” refers to the amount of a therapy (*e.g.*, prophylactic agent) which is sufficient to result in the prevention of the development, recurrence, or onset of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative

disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof), or to enhance or improve the prophylactic effect(s) of another therapy (*e.g.*, a prophylactic agent).

**[00120]** As used herein, a “prophylactic protocol” refers to a regimen for dosing and timing the administration of one or more therapies (*e.g.*, one or more prophylactic agents) that has a prophylactic effect.

**[00121]** As used herein, a “protocol” includes dosing schedules and dosing regimens. The protocols herein are methods of use and include prophylactic and therapeutic protocols.

**[00122]** As used herein, the phrase “side effects” encompasses unwanted and adverse effects of a prophylactic or therapeutic agent. Side effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a therapy (*e.g.*, a prophylactic or therapeutic agent) might be harmful, uncomfortable, or risky.

**[00123]** As used herein, the term “small molecules” and analogous terms include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such agents.

**[00124]** As used herein, the terms “subject” and “patient” are used interchangeably. As used herein, the terms “subject” and “subjects” refer to an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, and mouse) and a primate (*e.g.*, a monkey, such as a cynomolgous monkey, chimpanzee, and a human), and more preferably a human. In a certain embodiment, the subject is a mammal, preferably a human, with a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In another embodiment, the subject is a farm animal (*e.g.*, a horse, pig, or cow) or a pet (*e.g.*, a dog or cat) with a disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or

activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In another embodiment, the subject is a mammal (*e.g.*, an immunocompromised or immunosuppressed mammal), preferably a human, at risk of developing a disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In another embodiment, the subject is not an immunocompromised or immunosuppressed mammal, preferably a human. In another embodiment, the subject is a mammal, preferably a human, with a lymphocyte count that is not under approximately 500 cells/mm<sup>3</sup>. In another embodiment, the subject is a human infant or a human infant born prematurely. In another embodiment, the subject is a human child or a human adult. In another embodiment, the subject is a human child with bronchopulmonary dysplasia, congenital heart diseases, or cystic fibrosis. In another embodiment, the subject is an elderly human. In yet another embodiment, the subject is a human in an institution or group home, such as, but not limited to, a nursing home.

**[00125]** As used herein, the term "synergistic" refers to a combination of therapies (*e.g.*, prophylactic or therapeutic agents) which is more effective than the additive effects of any two or more single therapies (*e.g.*, one or more prophylactic or therapeutic agents). A synergistic effect of a combination of therapies (*e.g.*, a combination of prophylactic or therapeutic agents) permits the use of lower dosages of one or more of therapies (*e.g.*, one or more prophylactic or therapeutic agents) and/or less frequent administration of said therapies to a subject with a respiratory condition. The ability to utilize lower dosages of therapies (*e.g.*, prophylactic or therapeutic agents) and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention or treatment of a respiratory condition. In addition, a synergistic effect can result in improved efficacy of therapies (*e.g.*, prophylactic or therapeutic agents) in the prevention or treatment of a respiratory condition. Finally, the synergistic effect of a combination of therapies (*e.g.*,

prophylactic or therapeutic agents) may avoid or reduce adverse or unwanted side effects associated with the use of any single therapy.

**[00126]** As used herein, the term “T cell receptor modulator” refers to an agent which modulates the phosphorylation of a T cell receptor, the activation of a signal transduction pathway associated with a T cell receptor and/or the expression of a particular protein associated with T cell receptor activity such as a cytokine. Such an agent may directly or indirectly modulate the phosphorylation of a T cell receptor, the activation of a signal transduction pathway associated with a T cell receptor, and/or the expression of a particular protein associated with T cell receptor activity such as a cytokine. Examples of T cell receptor modulators include, but are not limited to, peptides, polypeptides, proteins, fusion proteins and antibodies which immunospecifically bind to a T cell receptor or a fragment thereof. Further, examples of T cell receptor modulators include, but are not limited to, proteins, peptides, polypeptides (*e.g.*, soluble T cell receptors), fusion proteins and antibodies that immunospecifically bind to a ligand for a T cell receptor or fragments thereof.

**[00127]** As used herein, the terms “therapeutic agent” and “therapeutic agents” refer to any agent(s) which can be used in the prevention, treatment, management, or amelioration of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In certain embodiments, the term “therapeutic agent” refers to an antibody that binds to an IL-9 polypeptide. In certain other embodiments, the term “therapeutic agent” refers to an agent other than an antibody that immunospecifically binds to an IL-9 polypeptide. Preferably, a therapeutic agent is an agent that is known to be useful for, or has been or is currently being used for the prevention, treatment, management, or amelioration of such a disease or disorder. Therapeutic agents may be characterized as different agents based upon one or more effects the agents have *in vivo* and/or *in vitro*, for example, an anti-inflammatory agent may also be characterized as an immunomodulatory agent.

**[00128]** As used herein, the term “therapeutically effective amount” refers to the amount of a therapy (*e.g.*, an antibody that immunospecifically binds to an IL-9 polypeptide), that is sufficient to reduce the severity of a disease or disorder (*e.g.*, a disease

or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof), reduce the duration of a respiratory condition, ameliorate one or more symptoms of such a disease or disorder, prevent the advancement of such a disease or disorder, cause regression of such a disease or disorder, or enhance or improve the therapeutic effect(s) of another therapy.

**[00129]** The terms “therapies” and “therapy” can refer to any protocol(s), method(s), and/or agent(s) that can be used in the prevention, treatment, management, or amelioration of a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof). In certain embodiments, the terms “therapy” and “therapy” refer to anti-viral therapy, anti-bacterial therapy, anti-fungal therapy, biological therapy, supportive therapy, and/or other therapies useful in treatment, management, prevention, or amelioration of such a disease or disorder or one or more symptoms known to skilled medical personnel.

**[00130]** As used herein, the term “therapeutic protocol” refers to a regimen for dosing and timing the administration of one or more therapies (*e.g.*, therapeutic agents) that has a therapeutic effective.

**[00131]** As used herein, the terms “treat,” “treatment,” and “treating” refer to the reduction or amelioration of the progression, severity, and/or duration of such a disease or disorder (*e.g.*, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection)) or the amelioration of one or more symptoms thereof resulting from the administration of one or more therapies (including, but not limited to, the administration of one or more prophylactic or therapeutic agents). In certain embodiments, such terms refer to a reduction in the swelling of organs or tissues, or a reduction in the pain associated with a respiratory condition. In other embodiments, such terms refer to a reduction in the

inflammation or constriction of an airway(s) associated with asthma. In other embodiments, such terms refer to a reduction in the replication of an infectious agent, or a reduction in the spread of an infectious agent to other organs or tissues in a subject or to other subjects. In other embodiments, such terms refer to the reduction of the release of inflammatory agents by mast cells, or the reduction of the biological effect of such inflammatory agents. In other embodiments, such terms refer to a reduction of the growth, formation and/or increase in the number of hyperproliferative cells (*e.g.*, cancerous cells). In yet other embodiments, such terms refer to the eradication, removal or control of primary, regional or metastatic cancer (*e.g.*, the minimization or delay of the spread of cancer).

#### **4. BRIEF DESCRIPTION OF THE DRAWINGS**

**[00132]** FIGS. 1A-B show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 7) of 4D4 with the VH CDR1 (SEQ ID NO.: 1), the VH CDR2 (SEQ ID NO.: ~~261~~), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left; and (B) variable light domain (SEQ ID. NO.: 8) of 4D4, with the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 5), and the VL CDR3 (SEQ ID NO.: 6) underlined, starting in order from VL CDR1 at the far left.

**[00133]** FIGS. 2A-B show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 9) of 4D4 H2-1 D11, with the VH CDR1 (SEQ ID NO.: 1), the VH CDR2 (SEQ ID NO.: 10), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left; and (B) variable light domain (SEQ ID. NO.: 8) of 4D4 H2-1 D11, the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 5), and the VL CDR3 (SEQ ID NO.: 6) underlined, starting in order from VL CDR1 at the far left.

**[00134]** FIGS. 3A-B show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 15) of 4D4com-XF-9, with the VH CDR1 (SEQ ID NO.: 11), the VH CDR2 (SEQ ID NO.: 10), and the VH CDR3 (SEQ ID NO.: 12) underlined, starting in order from VH CDR1 at the far left; and (B) variable light domain (SEQ ID. NO.: 16) of 4D4com-XF-9, the VL CDR1 (SEQ ID NO.: 13), the VL CDR2 (SEQ ID NO.: 14), and the VL CDR3 (SEQ ID NO.: ~~663~~) underlined, starting in order from VL CDR1 at the far left.

**[00135]** FIGS. 4A-B show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 17) of 4D4com-2F9, with the VH CDR1 (SEQ ID NO.: 1), the VH CDR2 (SEQ ID NO.: 10), and the VH CDR3 (SEQ ID NO.: 12) underlined, starting in



order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID. NO.: 18) of 4D4com-2F9, with the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 14), and the VL CDR3 (SEQ ID NO.: 664) underlined, starting in order from VL CDR1 at the far left.

**[00136]**        **FIGS. 5A-B** show the amino acid sequences of the **(A)** variable heavy domain (SEQ ID NO.: 21) of 7F3, with the VH CDR1 (SEQ ID NO.: 19), the VH CDR2 (SEQ ID NO.: 261), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID. NO.: 22) of 7F3, with the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 5), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

**[00137]**        **FIGS. 6A-B** show the amino acid sequences of the **(A)** variable heavy domain (SEQ ID NO.: 23) of 71A10, with the VH CDR1 (SEQ ID NO.: 19), the VH CDR2 (SEQ ID NO.: 2), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID. NO.: 24) of 71A10, the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 5), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

**[00138]**        **FIGS. 7A-B** show the amino acid sequences of the **(A)** variable heavy domain (SEQ ID NO.: 21) of 7F3 22D3, with the VH CDR1 (SEQ ID NO.: 19), the VH CDR2 (SEQ ID NO.: 261), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID. NO.: 25) of 7F3 22D3, with the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 14), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

**[00139]**        **FIGS. 8A-B** show the amino acid sequences of the **(A)** variable heavy domain (SEQ ID NO.: 27) of 7F3com-2H2, the VH CDR1 (SEQ ID NO.: 26), with the VH CDR2 (SEQ ID NO.: 2), and the VH CDR3 (SEQ ID NO.: 3) are underlined, starting in order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID. NO.: 28) of 7F3com-2H2, the VL CDR1 (SEQ ID NO.: 462), the VL CDR2 (SEQ ID NO.: 4465), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

**[00140]**        **FIGS. 9A-B** show the nucleotide sequences of the **(A)** variable heavy domain (SEQ ID NO.: 43) of 7F3com-2H2 with the VH CDR1 (SEQ ID NO.: 44), the VH CDR2 (SEQ ID NO.: 45) and the VH CDR3 (SEQ ID NO.: 46) underlined, starting in order from VH CDR1 at the far left; and **(B)** variable light domain (SEQ ID NO.: 47) of 7F3com-

2H2 with the VL CDR1 (SEQ ID NO.: 48), the VL CDR2 (SEQ ID NO.:49), and the VL CDR3 (SEQ ID NO.: 50) underlined, starting in order from VL CDR1 at the far left.

[00141] **FIGS. 10A-B** show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 29) of 7F3com-3H5, with the VH CDR1 (SEQ ID NO.: 19), the VH CDR2 (SEQ ID NO.: 2), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left and (B) variable light domain (SEQ ID. NO.: 30) of 7F3com-3H5, with the VL CDR1 (SEQ ID NO.: 4), the VL CDR2 (SEQ ID NO.: 14), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

[00142] **FIGS. 11A-B** show the amino acid sequences of the (A) variable heavy domain (SEQ ID NO.: 31) of 7F3com-3D4, with the VH CDR1 (SEQ ID NO.: 26), the VH CDR2 (SEQ ID NO.: 2), and the VH CDR3 (SEQ ID NO.: 3) underlined, starting in order from VH CDR1 at the far left and (B) variable light domain (SEQ ID. NO.: 32) of 7F3com-3D4, with the VL CDR1 (SEQ ID NO.:462), the VL CDR2 (SEQ ID NO.: 14), and the VL CDR3 (SEQ ID NO.: 20) underlined, starting in order from VL CDR1 at the far left.

[00143] **FIG. 12** shows the nucleotide sequence of human IL-9 (SEQ ID NO.: 51) located in the GenBank database (Accession Nos. NM\_000590).

[00144] **FIG. 13** shows the amino acid sequence for human IL-9 located in the GenBank database (Accession Nos. A60480 (SEQ ID NO.: 52), NP\_000584 (SEQ ID NO.: 53) and AAC17735 (SEQ ID NO.: 54)).

[00145] **FIGS. 14A-C** shows the nucleotide sequence of human IL-9R subunits found in the GenBank database (Accession Nos. NM\_002186 (SEQ ID NO.: 55), NM\_176786 (SEQ ID NO.: 56), and NM\_000206 (SEQ ID NO.: 57)). (A) Accession No. NM\_002186 and (B) Accession No. NM\_176786 are the nucleotide sequences of human IL-9R alpha subunit isoform precursors. (C) Accession No. NM\_000206 is the nucleotide sequence of the human IL-9R gamma chain.

[00146] **FIG. 15** shows the amino acid sequence of human IL-9R found in the GenBank database (Accession Nos. NP\_002177 (SEQ ID NO.: 58); NP\_789743 (SEQ ID NO.: 59), and NP\_000197 (SEQ ID NO.: 60)). Accession Nos. NP\_002177 and NP\_789743 are the amino acid sequences of human IL-9R alpha subunit isoform precursors. NP\_000197 is the amino acid sequence of the human IL-9R gamma chain.

## **5. DETAILED DESCRIPTION OF THE INVENTION**

**[00147]** The present invention provides antibodies that immunospecifically bind to an interleukin-9 (“IL-9”) polypeptide (preferably, a human IL-9 polypeptide). In particular, the invention provides the following antibodies that immunospecifically bind to an IL-9 polypeptide: 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. The present invention also provides for antibodies comprising a variable heavy (“VH”) domain and/or a variable light (“VL”) domain having an amino acid sequence of the VH domain and/or VL domain, respectively, of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. Such antibodies may further comprise any constant region known in the art, preferably any human constant region known in the art, including, but not limited to, human light chain kappa ( $\kappa$ ), human light chain lambda ( $\lambda$ ), the constant region of IgG<sub>1</sub>, the constant region of IgG<sub>2</sub>, the constant region of IgG<sub>3</sub> or the constant region of IgG<sub>4</sub>. In addition, the present invention provides for antibodies comprising one or more complementarity determining regions (“CDRs”) of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4.

**[00148]** The present invention encompasses treatment protocols that provide better prophylactic or therapeutic profiles than current single agent therapies or combination therapies for diseases or disorders associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders associated with or characterized by aberrant expression and/or activity of the IL-9 receptor (“IL-9R”) or one or more subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, or infections (preferably, respiratory infections), or one or more symptoms thereof. In particular, the invention provides prophylactic and therapeutic protocols for the prevention, treatment, management, and/or amelioration of diseases or disorders characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, or infections (preferably, respiratory infections), or one or more symptoms thereof, comprising administering to a subject an effective amount of one or more of the antibodies of the invention alone or in combination with an effective amount of at least one therapy (*e.g.*, a prophylactic or therapeutic agent) other than an antibody of the invention.

**[00149]** The present invention provides for pharmaceutical compositions, kits, and articles of manufacture comprising one or more antibodies that immunospecifically binds to an IL-9 polypeptide for use in the prevention, treatment, management, and/or amelioration of a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection), or one or more symptoms thereof. The present invention also provides for pharmaceutical compositions, kits, and articles of manufacture comprising one or more antibodies that immunospecifically bind to an IL-9 polypeptide and one or more prophylactic or therapeutic agents other than antibodies of the invention for use in the prevention, treatment, management, or amelioration of a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of the IL-9 receptor or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection), or one or more symptoms thereof.

## **5.1 IL-9 ANTIBODIES**

**[00150]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide (preferably, a human IL-9 polypeptide). In particular, the invention provides the following antibodies that immunospecifically bind to an IL-9 polypeptide: 4D4 or an antigen-binding fragment thereof, 4D4 H2-1 D11 or an antigen-binding fragment thereof, 4D4com-XF-9 or an antigen-binding fragment thereof, 4D4com-2F9 or an antigen-binding fragment thereof, 7F3 or an antigen-binding fragment thereof, 71A10 or an antigen-binding fragment thereof, 7F3 22D3 or an antigen-binding fragment thereof, 7F3com-2H2 or an antigen-binding fragment thereof, 7F3com-3H5 or an antigen-binding fragment thereof, and 7F3com-3D4 or an antigen-binding fragment thereof. In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide is 7F3com-2H2 or an antigen-binding fragment thereof (*e.g.*, one or more CDRs of 7F3com-2H2). The constant regions for 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 71A10, 7F3 22D3, 7F3com, 7F3com-2H2, 7F3com-3H5, and 7F3com-3D4 are identical to the constant regions of palivizumab (MedImmune, Inc.) IgG<sub>1</sub> (see U.S. Pat. No. 5,824,307, issued October 20, 1998).

**[00151]** The present invention provides antibodies that immunospecifically bind an IL-9 polypeptide, said antibodies comprising a VH domain having an amino acid sequence of the VH domain of 4D4 (FIG. 1A; SEQ ID NO.: 7), 4D4 H2-1 D11 (FIG. 2A; SEQ ID NO.: 9), 4D4com-XF-9 (FIG. 3A; SEQ ID NO.: 15), 4D4com-2F9 (FIG. 4A; SEQ ID NO.: 17), 7F3 (FIG. 5A; SEQ ID NO.: 21), 71A10 (FIG. 6A; SEQ ID NO.: 23), 7F3 22D3 (FIG. 7A; SEQ ID NO.: 21), 7F3com-2H2 (FIG. 8A; SEQ ID NO.: 27), 7F3com-3H5 (FIG. 10A; SEQ ID NO.: 29), or 7F3com-3D4 (FIG. 11A; SEQ ID NO.: 31). In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH domain having an amino acid sequence of the VH domain of 7F3com-2H2 (FIG. 8A; SEQ ID NO.: 27).

**[00152]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 1, *infra*. In particular, the invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising (or alternatively, consisting of) one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 1, *infra*. In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26 and a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26 and a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61 and a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12.

NO.: 12. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26, a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61, and a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12.

**[00153]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VL domain having an amino acid sequence of the VL domain for 4D4 (FIG. 1B; SEQ ID NO.: 8), 4D4 H2-1 D11 (FIG. 2B; SEQ ID NO.: 8), 4D4com-XF-9 (FIG. 3B; SEQ ID NO.: 16), 4D4com-2F9 (FIG. 4B; SEQ ID NO.: 18), 7F3 (FIG. 5B; SEQ ID NO.: 22), 71A10 (FIG. 6B; SEQ ID NO.: 24), 7F3 22D3 (FIG. 7B; SEQ ID NO.: 25), 7F3com-2H2 (FIG. 8B; SEQ ID NO.: 28), 7F3com-3H5 (FIG. 10B; SEQ ID NO.: 30), or 7F3com-3D4 (FIG. 11B; SEQ ID NO.: 32). In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL domain having an amino acid sequence of the VL domain for 7F3com-2H2 (FIG. 8B; SEQ ID NO.: 28).

**[00154]** The present invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 1, *infra*. In particular, the invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising (or alternatively, consisting of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 1, *infra*. In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR3 having the amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64. In another embodiment, an antibody of that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62 and a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65. In another embodiment of an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR1 having the amino acid sequence of SEQ ID

NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62 and a VL CDR3 having the amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65 and a VL CDR3 having the amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62, a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65, and a VL CDR3 having the amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64, being a part of the antibody.

**Table 1.**

Residues that are different between each amino acid sequence encoding the various CDRs appear in bold, underlined font.

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
4D4	SEQ. ID NO.: 7	GYTFTIGYWI E (SEQ. ID NO. 1):	EILPGSGTTN YNEKFKG (SEQ. ID NO.: 261)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 8	<b>K</b> ASQHVGVTH VT (SEQ. ID NO.: 4)	<b>S</b> TSYRYS (SEQ. ID NO.: 5)	<b>Q</b> HFYSYPLT (SEQ. ID NO.: 6)
	SEQ. ID NO.: 9	GYTFTIGYWI E (SEQ. ID NO. 1)	EWLPGSGTT NYNEKFKG (SEQ. ID NO.: 10)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 8	<b>K</b> ASQHVGVTH VT (SEQ. ID NO.: 4)	<b>S</b> TSYRYS (SEQ. ID NO.: 5)	<b>Q</b> HFYSYPLT (SEQ. ID NO.: 6)
4D4com-XF-9	SEQ. ID NO.: 15	GYTFTIGYWI E (SEQ. ID NO. 11)	EWLPGSGTT NYNEKFKG (SEQ. ID NO.: 10)	ADYYGSDHV KFDY (SEQ. ID NO.: 12)	SEQ. ID NO.: 16	<b>L</b> ASQHVGVTH VT (SEQ. ID NO.: 13)	<b>G</b> TSYRYS (SEQ. ID NO.: 14)	<b>Q</b> HFYDYPLT (SEQ. ID NO.: 663)
	SEQ. ID NO.: 17	GYTFTIGYWI E (SEQ. ID NO. 1)	EWLPGSGTT NYNEKFKG (SEQ. ID NO.: 10)	ADYYGSDHV KFDY (SEQ. ID NO.: 12)	SEQ. ID NO.: 18	<b>K</b> ASQHVGVTH VT (SEQ. ID NO.: 4)	<b>G</b> TSYRYS (SEQ. ID NO.: 14)	<b>Q</b> HFYDYPLT (SEQ. ID NO.: 664)
7F3	SEQ. ID NO.: 21	GGTFSGYWI E (SEQ. ID NO. 19)	EILPGSGTTN YNEKFKG (SEQ. ID NO.: 261)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 22	<b>K</b> ASQHVGVTH VT (SEQ. ID NO.: 4)	<b>S</b> TSYRYS (SEQ. ID NO.: 5)	<b>Q</b> QFYDYPLT (SEQ. ID NO.: 20)
	SEQ. ID NO.: 23	GGTFSGYWI E (SEQ. ID NO. 19)	EILPGSGTTN PNEKFKG (SEQ. ID NO.: 2)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 24	<b>K</b> ASQHVGVTH VT (SEQ. ID NO.: 4)	<b>S</b> TSYRYS (SEQ. ID NO.: 5)	<b>Q</b> QFYDYPLT (SEQ. ID NO.: 20)



**Table 1 continued.**

Residues that are different between each amino acid sequence encoding the various CDRs appear in bold, underlined font.

7F3 22D3	SEQ. ID NO.: 21	GGTF <u><b>S</b></u> GYWI E (SEQ. ID NO.: 19)	EILPGSGTTN YNEKFKG (SEQ. ID NO.: <u>261</u> )	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 25	<u><b>K</b></u> ASQHVGVTH VT (SEQ. ID NO.: 4)	<u><b>G</b></u> TSYRYS (SEQ. ID NO.: 14)	<u><b>Q</b></u> QFYEYPLT (SEQ. ID NO.: 20)
7F3com-2H2	SEQ. ID NO.: 27	GGTF <u><b>S</b></u> YGYWI E (SEQ. ID NO.: 26)	EILPGSGTTN PNEKFKG (SEQ. ID NO.: 2)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 28	<u><b>K</b></u> ASQHVITH VT (SEQ. ID NO.: <u>462</u> )	<u><b>G</b></u> TSYSYS (SEQ. ID NO.: <u>1465</u> )	<u><b>Q</b></u> QFYEYPLT (SEQ. ID NO.: 20)
7F3com-3H5	SEQ. ID NO.: 29	GGTF <u><b>S</b></u> GYWI E (SEQ. ID NO.: 19)	EILPGSGTTN PNEKFKG (SEQ. ID NO.: 2)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 30	<u><b>K</b></u> ASQHVGVTH VT (SEQ. ID NO.: 4)	<u><b>G</b></u> TSYRYS (SEQ. ID NO.: 14)	<u><b>Q</b></u> QFYEYPLT (SEQ. ID NO.: 20)
7F3com-3D4	SEQ. ID NO.: 31	GGTF <u><b>S</b></u> YGYWI E (SEQ. ID NO.: 26)	EILPGSGTTN PNEKFKG (SEQ. ID NO.: 2)	ADYYGSDYV KFDY (SEQ. ID NO.: 3)	SEQ. ID NO.: 32	<u><b>K</b></u> ASQHVITH VT (SEQ. ID NO.: <u>462</u> )	<u><b>G</b></u> TSYRYS (SEQ. ID NO.: 14)	<u><b>Q</b></u> QFYEYPLT (SEQ. ID NO.: 20)

**[00155]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VH domain disclosed herein combined with a VL domain disclosed herein, or other VL domain (*e.g.*, a VL domain disclosed in U.S. provisional application Serial No. 60/371,683, filed April 12, 2002 and U.S. provisional application Serial No. 60/371,728, filed April 12, 2002, each of which is incorporated herein by reference in its entirety). The present invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a VL domain disclosed herein combined with a VH domain disclosed herein, or other VH domain (*e.g.*, a VH domain disclosed in U.S. provisional application Serial No. 60/371,683, filed April 12, 2002 and U.S. provisional application Serial No. 60/371,728, filed April 12, 2002).

**[00156]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising (or alternatively, consisting of) a VH CDR listed in Table 1, *supra* and a VL CDR disclosed in U.S. provisional application Serial No. 60/371,683, filed April 12, 2002 and U.S. provisional application Serial No. 60/371,728, filed April 12, 2002. The present invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising (or alternatively, consisting of) a VL CDR listed in Table 1, *supra* and a VH CDR disclosed in U.S. provisional application Serial No. 60/371,683, filed April 12, 2002 and U.S. provisional application Serial No. 60/371,728, filed April 12, 2002. The invention further provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising combinations of VH CDRs and VL CDRs described herein and disclosed in U.S. provisional application Serial No. 60/371,683, filed April 12, 2002 and U.S. provisional application Serial No. 60/371,728, filed April 12, 2002.

**[00157]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising one or more VH CDRs and one or more VL CDRs listed in Table 1, *supra*. In particular, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (or alternatively, consisting of) a VH CDR1 and a VL CDR1; a VH CDR1 and a VL CDR2; a VH CDR1 and a VL CDR3; a VH CDR2 and a VL CDR1; VH CDR2 and VL CDR2; a VH CDR2 and a VL CDR3; a VH CDR3 and a VH CDR1; a VH CDR3 and a VL CDR2; a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR2 and a VL CDR1; a VH CDR1, a VH CDR2 and a VL CDR2; a VH CDR1, a VH CDR2 and a VL CDR3; a VH CDR2, a VH CDR3 and a VL CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR2, a VH CDR2 and a VL CDR3; a VH CDR1, a VL CDR1 and a VL CDR2; a VH CDR1, a VL

CDR1 and a VL CDR3; a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR1; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; or any combination thereof of the VH CDRs and VL CDRs listed in Table 1, *supra*.

**[00158]** In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26 and a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26 and a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR1 having the amino acid sequence of SEQ ID NO.: 1, SEQ ID NO.: 11, SEQ ID NO.: 19, or SEQ ID NO.: 26 and a VL CDR3 having an amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64.

**[00159]** In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61 and a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR2 having the amino acid sequence of SEQ ID NO.: 2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61 and a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5, ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR2 having the amino acid sequence of SEQ ID NO.:

2, ~~or~~ SEQ ID NO.: 10 or SEQ ID NO.: 61 and a VL CDR3 having an amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64.

[00160] In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12 and a VL CDR1 having the amino acid sequence of SEQ ID NO.: 4, ~~or~~ SEQ ID NO.: 13 or SEQ ID NO.: 62. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12 and a VL CDR2 having the amino acid sequence of SEQ ID NO.: 5 ~~or~~ SEQ ID NO.: 14 or SEQ ID NO.: 65. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR3 having the amino acid sequence of SEQ ID NO.: 3 or SEQ ID NO.: 12 and a VL CDR3 having an amino acid sequence of SEQ ID NO.: 6, ~~or~~ SEQ ID NO.: 20, SEQ ID NO.: 63 or SEQ ID NO.: 64.

[00161] The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies encoded by a nucleic acid sequence comprising the nucleotide sequence of 7F3com-2H2 or an antigen-binding fragment thereof. In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH domain encoded by a nucleic acid sequence having a nucleotide sequence of the VH domain of 7F3com-2H2. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL domain encoded by a nucleic acid sequence having a nucleotide sequence of the VL domain of 7F3com-2H2. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH domain and a VL domain encoded by a nucleic acid sequence having a nucleotide sequence of the VH domain and VL domain of 7F3com-2H2.

[00162] In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR encoded by a nucleic acid sequence having a nucleotide sequence of a VH CDR of 7F3com-2H2. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VL CDR encoded by a nucleic acid sequence having a nucleotide sequence of a VL CDR of 7F3com-2H2. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a VH CDR and a VL CDR encoded by a nucleic acid sequence having a nucleotide sequence of a VH CDR and a VL CDR of 7F3com-2H2.

[00163] The present invention provides for a nucleic acid molecule, generally isolated, encoding an antibody of the present invention that immunospecifically binds to an IL-9 polypeptide. In particular, the invention provides an isolated nucleic acid molecule

encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody having the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, or an antigen-binding fragment thereof. In a preferred embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody having the amino acid sequence of 7F3com-2H2.

**[00164]** The invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (alternatively, consisting of) a VH domain having an amino acid sequence of a VH domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. In a preferred embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH domain having the amino acid sequence of the VH domain of 7F3com-2H2.

**[00165]** The invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (alternatively, consisting of) a VH CDR having an amino acid sequence of any of the VH CDRs listed in Table 1, *supra*. In particular, the invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 1, *supra*. In one embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH CDR1 having the amino acid sequence of the VH CDR1 listed in Table 1, *supra*. In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH CDR2 having the amino acid sequence of the VH CDR2 listed in Table 1, *supra*. In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH CDR3 having the amino acid sequence of the VH CDR3 listed in Table 1, *supra*.

**[00166]** The invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (alternatively, consisting of) a VL domain having an amino acid sequence of a VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4. In a preferred embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said

antibody comprising a VL domain having the amino acid sequence of the VL domain of 7F3com-2H2.

**[00167]** The invention also provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (alternatively, consisting of) a VL CDR having an amino acid sequence of any of the VL CDRs listed in Table 1, *supra*. In particular, the invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 1, *supra*. In one embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VL CDR1 having the amino acid sequence of the VH CDR1 listed in Table 1, *supra*. In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VL CDR2 having the amino acid sequence of the VL CDR2 listed in Table 1, *supra*. In another embodiment, an isolated nucleic acid molecule encodes an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VL CDR3 having the amino acid sequence of the VL CDR3 listed in Table 1, *supra*.

**[00168]** The present invention provides nucleic acid molecules encoding antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising one or more VH CDRs and one or more VL CDRs listed in Table 1, *supra*. In particular, the invention provides an isolated nucleic acid molecule encoding an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising (or alternatively, consisting of) a VH CDR1 and a VL CDR1; a VH CDR1 and a VL CDR2; a VH CDR1 and a VL CDR3; a VH CDR2 and a VL CDR1; VH CDR2 and VL CDR2; a VH CDR2 and a VL CDR3; a VH CDR3 and a VH CDR1; a VH CDR3 and a VL CDR2; a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR2 and a VL CDR1; a VH CDR1, a VH CDR2 and a VL CDR2; a VH CDR1, a VH CDR2 and a VL CDR3; a VH CDR2, a VH CDR3 and a VL CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR2, a VH CDR2 and a VL CDR3; a VH CDR1, a VL CDR1 and a VL CDR2; a VH CDR1, a VL CDR1 and a VL CDR3; a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR1; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1 and a VL

CDR2; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; or any combination thereof of the VH CDRs and VL CDRs listed in Table 1, *supra*.

**[00169]** The present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising derivatives of the VH domains, VH CDRs, VL domains, or VL CDRs described herein that immunospecifically bind to an IL-9 polypeptide. Standard techniques known to those of skill in the art can be used to introduce mutations (*e.g.*, deletions, additions, and/or substitutions) in the nucleotide sequence encoding an antibody of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Preferably, the derivatives include less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original molecule. In a preferred embodiment, the derivatives have conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues (*i.e.*, amino acid residues which are not critical for the antibody to immunospecifically bind to an IL-9 polypeptide). A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded antibody can be expressed and the activity of the antibody can be determined.

**[00170]** The present invention provides for antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4 with one or more amino acid residue substitutions in the variable light (VL) domain and/or variable heavy (VH) domain. The present invention also provides for antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4 with one or more amino acid residue substitutions in one or more VL CDRs and/or one or more VH CDRs. The present invention also provides for antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4, or a VH and/or VL domain thereof with one or more amino acid residue substitutions in one or more VH frameworks and/or one or more VL frameworks. The antibody generated by introducing substitutions in the VH domain, VH CDRs, VL domain, VL CDRs and/or frameworks of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5, or 7F3com-3D4 can be tested *in vitro* and/or *in vivo*, for example, for its ability to bind to an IL-9 polypeptide, or for its ability to inhibit or reduce IL-9 mediated cell proliferation, or for its ability to prevent, treat and/or ameliorate an autoimmune disorder, an inflammatory disorder, a proliferative disorder or a respiratory infection, or a symptom thereof.

**[00171]** In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises a nucleotide sequence that hybridizes to the nucleotide sequence encoding 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, or an antigen-binding fragment thereof under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65°C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45°C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68°C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. *et al.*, eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3).

**[00172]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VH domain or an amino acid



sequence a VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding the VH or VL domains of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 under stringent conditions described herein or under other stringent hybridization conditions which are known to those of skill in the art. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VH domain and an amino acid sequence of a VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding the VH and VL domains of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 under stringent conditions described herein or under other stringent hybridization conditions which are known to those of skill in the art. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VH CDR or an amino acid sequence of a VL CDR encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding any one of the VH CDRs or VL CDRs listed in Table 1, *supra* under stringent conditions described herein or under other stringent hybridization conditions which are known to those of skill in the art. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VH CDR and an amino acid sequence of a VL CDR encoded by nucleotide sequences that hybridize to the nucleotide sequences encoding any one of the VH CDRs listed in Table 1, *supra*, and any one of the VL CDRs listed Table 1, *supra*, under stringent conditions described herein or under other stringent hybridization conditions which are known to those of skill in the art.

**[00173]** In another embodiment, the present invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH domain and/or VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence of the VH domain and/or VL domain of 7F3com-2H2 (SEQ ID NO.: 43 and SEQ ID NO.: 47, respectively) under stringent conditions. In another embodiment, the present invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH CDR and/or VL CDR encoded by a nucleotide sequence that hybridizes to the nucleotide sequence of the VH CDR and/or VL CDR of 7F3com-2H2 (FIGS. 9A-B) under stringent conditions.

**[00174]** In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence that is at least 35%, preferably at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to

the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, or an antigen-binding fragment thereof. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VH domain that is at least 35%, preferably at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the VH domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of a VL domain that is at least 35%, preferably at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4.

**[00175]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of one or more VL CDRs that are at least 35%, preferably at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any of the VL CDRs listed in Table 1, *supra*. In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide comprises an amino acid sequence of one or more VL-VH CDRs that are at least 35%, preferably at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any of one of the VL-VH CDRs listed in Table 1, *supra*.

**[00176]** In another embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody encoded by a nucleotide sequence that is at least 65%, preferably at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the nucleotide sequence encoding 7F3com-2H2. In another embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said antibody comprising a VH domain and/or VL domain encoded by a nucleotide sequence that is at least 65%, preferably at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the nucleotide sequence of the VH domain and/or VL domain of 7F3com-2H2 (SEQ ID NO.: 43 and SEQ ID NO.: 47, respectively). In another embodiment, the invention provides an antibody that immunospecifically binds to an IL-9 polypeptide, said

antibody comprising a VH CDR and/or a VL CDR encoded by a nucleotide sequence that is at least 65%, preferably at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the nucleotide sequence of the VH CDR and/or VL CDR of 7F3com-2H2 (FIGS. 9A-B).

**[00177]** The present invention encompasses antibodies that compete with an antibody described herein for binding to an IL-9 polypeptide. In particular, the present invention encompasses antibodies that compete with 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 or an antigen-binding fragment thereof for binding to the IL-9 polypeptide. In a specific embodiment, the invention encompasses an antibody that reduces the binding of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 to an IL-9 polypeptide by at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in the competition assay described herein or competition assays well known in the art. In another embodiment, the invention encompasses an antibody that reduces binding of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 to an IL-9 polypeptide by at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, or 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in an ELISA competition assay. In a preferred embodiment, an ELISA competition assay may be performed in the following manner: recombinant IL-9 is prepared in PBS at a concentration of 10 µg/ml. 100 µl of this solution is added to each well of an ELISA 96-well microtiter plate and incubated overnight at 4-8°C. The ELISA plate is washed with PBS supplemented with 0.1% Tween to remove excess recombinant IL-9. Non-specific protein-protein interactions are blocked by adding 100 µl of bovine serum albumin (BSA) prepared in PBS to a final concentration of 1%. After one hour at room temperature, the ELISA plate is washed. Unlabeled competing antibodies are prepared in blocking solution at concentrations ranging from 1 µg/ml to 0.01 µg/ml. Control wells contain either blocking solution only or control antibodies at concentrations ranging from 1 µg/ml to 0.01 µg/ml. Test antibody (*e.g.*, 7F3com-2H2) labeled with horseradish peroxidase is added to competing antibody dilutions at a fixed final concentration of 1 µg/ml. 100 µl of test and competing antibody mixtures are added to the ELISA wells in triplicate and the plate is incubated for 1 hour at room temperature.

Residual unbound antibody is washed away. Bound test antibody is detected by adding 100 µl of horseradish peroxidase substrate to each well. The plate is incubated for 30 min. at room temperature, and absorbance is read using an automated plate reader. The average of triplicate wells is calculated. Antibodies which compete well with the test antibody reduce the measured absorbance compared with control wells. In a preferred embodiment, the invention encompasses an antibody that reduces the binding of 7F3com-2H2 to an IL-9 polypeptide by at least 25%, at least 30%, at least 35%, at least 40 %, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, or 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in an ELISA competition assay.

**[00178]** In another embodiment, the invention encompasses an antibody that reduces the binding of an antibody comprising (alternatively, consisting of) an antigen-binding fragment (*e.g.*, a VH domain, a VH CDR, a VL domain or a VL CDR) of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 to an IL-9 polypeptide by at least 25%, preferably at least 30%, at least 35%, at least 40 %, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, or 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in a competition assay described herein or well-known to one of skill in the art. In another embodiment, the invention encompasses an antibody that reduces the binding of an antibody comprising (alternatively, consisting of) an antigen-binding fragment (*e.g.*, a VH domain, VL domain, a VH CDR, or a VL CDR) of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 to an IL-9 polypeptide by at least 25%, preferably at least 30%, at least 35%, at least 40 %, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, or 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in an ELISA competition assay. In a preferred embodiment, the invention encompasses an antibody that reduces the binding of an antibody comprising (alternatively, consisting of) an antigen-binding fragment of 7F3com-2H2 to an IL-9 polypeptide by at least 25%, preferably at least 30%, at least 35%, at least 40 %, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more, or 25% to 50%, 45 to 75%, or 75 to 99% relative to a control such as PBS in an ELISA competition assay.

**[00179]** The present invention encompasses polypeptides or proteins comprising (alternatively, consisting of) VH domains that compete with the VH domain of 4D4, 4D4

H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 for binding to an IL-9 polypeptide. The present invention also encompasses polypeptides or proteins comprising (alternatively, consisting of) VL domains that compete with a VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 for binding to an IL-9 polypeptide.

**[00180]** The present invention encompasses polypeptides or proteins comprising (alternatively, consisting of) VH CDRs that compete with a VH CDR listed in Table 1, *supra*, for binding to an IL-9 polypeptide. The present invention also encompasses polypeptides or proteins comprising (alternatively, consisting of) VL CDRs that compete with a VL CDR listed in Table 1, *supra* for binding to an IL-9 polypeptide.

**[00181]** The antibodies that immunospecifically bind to an IL-9 polypeptide include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, *etc.* Additionally, the derivative may contain one or more non-classical amino acids.

**[00182]** The present invention also provides antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising a framework region known to those of skill in the art (*e.g.*, a human or non-human framework). The framework regions may be naturally occurring or consensus framework regions. Preferably, the fragment region of an antibody of the invention is human (see, *e.g.*, Chothia *et al.*, 1998, J. Mol. Biol. 278:457-479 for a listing of human framework regions, which is incorporated herein by reference in its entirety).

**[00183]** The present invention encompasses antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 with mutations (*e.g.*, one or more amino acid substitutions) in the framework regions. In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide comprise the amino acid sequence of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 with

one or more amino acid residue substitutions in the framework regions of the VH and/or VL domains. Preferably, the amino acid substitutions in the framework region improve binding of the antibody to an IL-9 polypeptide.

**[00184]** In a specific embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide comprise the amino acid sequence of one or more of the CDRs of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, a VH framework region 1 having the amino acid sequence of QVQLVQSGAEVKKPGASVKVCKAS (SEQ ID NO.: 33) or QVQLVQSGAEV KKPGSSVKVCKAS (SEQ ID NO.: 37), a VH framework region 2 having the amino acid sequence of WVRQAPGQGLEWMG (SEQ ID NO.: 34), a VH framework region 3 region having the amino acid sequence of RVTMTRDTSTSTVYMELSSLRSED TAVYYCAR (SEQ ID NO.: 35) or RVTITADESTSTAYMELSSLRSED TAVYYCAR (SEQ ID NO.: 38), and a VH framework region 4 having the amino acid sequence of WGQGTLVTVSS (SEQ ID NO.: 36). In another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide comprise the amino acid sequence of one or more of the CDRs of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, a VL framework region 1 having the amino acid sequence of DIQMTQSPSSLSASVGDRVTITC (SEQ ID NO.: 39), a VL framework region 2 having the amino acid sequence of WYQQKPGKAPKLLIY (SEQ ID NO.: 40), a VL framework region 3 region having the amino acid sequence of GVPSRFGSGSGTDFTLTISSLQ PEDFATYYC (SEQ ID NO.: 41), and a VL framework region 4 region having the amino acid sequence of FGGGTKVEIK (SEQ ID NO.: 42). In yet another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide comprise the amino acid sequence of one or more of the CDRs of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, a VH framework region 1 having the amino acid sequence of SEQ ID NO.: 33 or SEQ ID NO.: 37, a VH framework region 2 having the amino acid sequence of SEQ ID NO.: 34, a VH framework region 3 having the amino acid sequence of SEQ ID NO.: 35 or SEQ ID NO.: 38, a VH framework region 4 having the amino acid sequence of SEQ ID NO.: 36, a VL framework region 1 having the amino acid sequence of SEQ ID NO.: 39, a VL framework region 2 having the amino acid sequence of SEQ ID NO.: 40, a VL framework region 3 having the amino acid sequence of SEQ ID NO.: 41, and a VL framework region 4 having the amino acid sequence of SEQ ID NO.: 42.

**[00185]** The present invention also encompasses antibodies that immunospecifically bind to an IL-9 polypeptide, said antibodies comprising the amino acid sequence of 4D4,

4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 with mutations (*e.g.*, one or more amino acid residue substitutions) in the variable and framework regions. Preferably, the amino acid substitutions in the variable and framework regions improve binding of the antibody to an IL-9 polypeptide.

**[00186]** The present invention also provides antibodies of the invention that comprise constant regions known to those of skill in the art. Preferably, the constant regions of an antibody of the invention or fragment thereof are human.

**[00187]** The invention encompasses antibodies that immunospecifically bind to an IL-9 polypeptide expressed by an immune cell such as an activated T cell or a mast cell. The invention also encompasses antibodies that immunospecifically bind to an IL-9 polypeptide and modulate an activity or function of T cells, B cells, mast cells, neutrophils, and/or eosinophils. The invention further encompasses antibodies that immunospecifically bind to an IL-9 polypeptide and inhibit or reduce the infiltration of inflammatory cells into a tissue, joint, or organ of a subject and/or inhibit or reduce epithelial cell hyperplasia.

**[00188]** The invention encompasses antibodies that immunospecifically bind to an IL-9 polypeptide found in the milieu, *i.e.*, not bound to an IL-9R or a subunit thereof. The invention also encompasses antibodies that immunospecifically bind to an IL-9 polypeptide bound to a soluble IL-9R $\alpha$  subunit. The invention further encompasses antibodies that immunospecifically bind to an IL-9 polypeptide bound to a cellular membrane-bound IL-9R or a subunit thereof.

**[00189]** In one embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide inhibit and/or reduce the interaction between the IL-9 polypeptide and the IL-9 receptor ("IL-9R") or a subunit thereof by approximately 25%, preferably approximately 30%, approximately 35%, approximately 45%, approximately 50%, approximately 55%, approximately 60%, approximately 65%, approximately 70%, approximately 75%, approximately 80%, approximately 85%, approximately 90%, approximately 95%, or approximately 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, an immunoassay such as an ELISA). In an alternative embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide does not inhibit the interaction between an IL-9 polypeptide and the IL-9R or a subunit thereof relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, an immunoassay such as an ELISA). In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide inhibits the interaction

between the IL-9 polypeptide and the IL-9R by less than 20%, less than 15%, less than 10%, or less than 5% relative to a control such as PBS or a control IgG antibody using, for example, an immunoassay such as an ELISA.

**[00190]** In one embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit or reduce the interaction between the IL-9 polypeptide and the IL-9 receptor ("IL-9R") or one or more subunits thereof by at least 25%, preferably, at least 30%, at least 35%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as phosphate buffered saline ("PBS") or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, a cell proliferation assay using an IL-9 dependent cell line such as an IL-9 dependent mouse T cell line expressing the human IL-9R). In an alternative embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide do not inhibit the interaction between an IL-9 polypeptide and the IL-9R or one or more subunits thereof relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, a cell proliferation assay using an IL-9 dependent cell line such as an IL-9 dependent mouse T cell line expressing the human IL-9R). In another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit the interaction between the IL-9 polypeptide and the IL-9R or one or more subunits thereof by less than 20%, less than 15%, less than 10%, or less than 5% relative to a control such as PBS or a control IgG antibody *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art, (*e.g.*, a cell proliferation assay using an IL-9 dependent cell line such as an IL-9 dependent mouse T cell line expressing the human IL-9R).

**[00191]** The present invention encompasses antibodies that immunospecifically bind to an IL-9 polypeptide and do not induce or reduce cytokine expression and/or release relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art. In one embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide and do not induce an increase in the concentration cytokines such as, *e.g.*, IFN- $\gamma$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, and IL-23 in the serum of a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. In an alternative embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide induce cytokine expression and/or release relative to a control such as PBS or a control IgG antibody in an *in vitro* and/or *in vivo*



assay described herein or well-known to one of skill in the art. In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide induces an increase in the concentration of cytokines such as, *e.g.*, IFN- $\gamma$ , IL-2, IL-12, and IL-15 in the serum of a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. In another specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide induces an increase in the concentration of cytokines produced by Th1 cells, such as IFN- $\gamma$  and IL-12, in a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. In another specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide induces a decrease in the concentration of cytokines such as, *e.g.*, IL-4, IL-5, IL-10, IL-13, and IL-23 in the serum of a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. In another specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide induces a decrease in the concentration of cytokines produced by mast cells, such as TNF- $\alpha$ , IL-4, and IL-13, in the serum of a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. In yet another specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide induces a decrease in the concentration of cytokines produced by Th2 cells, such as IL-4, IL-5, IL-13, and IL-10, in the serum of a subject administered such an antibody relative to the concentration of such cytokines in the serum of a subject administered a control such as PBS or a control IgG antibody. Serum concentrations of a cytokine can be measured by any technique well-known to one of skill in the art such as, *e.g.*, ELISA or Western blot assay.

**[00192]** In one embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide reduce and/or inhibit proliferation of inflammatory cells (*e.g.*, mast cells, T cells, B cells, macrophages, neutrophils, basophils, and/or eosinophils) by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, a trypan blue assay or  $^3\text{H}$ -thymidine assay). In another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide reduce and/or inhibit infiltration of inflammatory cells into the upper and/or lower respiratory tracts by at least at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%,

at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art. In yet another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide reduce and/or inhibit infiltration of inflammatory cells into the upper and/or respiratory tracts by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known in the art and reduce and/or inhibit proliferation of inflammatory cells by at least by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay or <sup>3</sup>H-thymidine assay).

**[00193]** In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide reduce mast cell degranulation by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (see, e.g., Windmiller and Backer, 2003, *J. Biol. Chem.* 278:11874-78 for examples of mast cell degranulation assays). In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce mast cell activation by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce the expression and/or release of products of mast cell activation and/or degranulation by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described or well-known to one of skill in the art.

**[00194]** In a specific embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce the expression, activity, serum concentration, and/or release of mast cell proteases, such as chymase and tryptase, by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art. In a preferred embodiment, mast cell activity may be measured by culturing primary mast cells or a mast cell line *in vitro* in the presence of 10 ng/ml of IL-9. Baseline levels of protease (*e.g.*, chymase and tryptase) and leukotriene are determined in the supernatant by commercially available ELISA kits. The ability of antibodies to modulate protease or leukotriene levels is assessed by adding an IL-9-reactive antibody or control antibody directly to cell cultures at a concentration of 1 µg/ml. Protease and leukotriene levels are assessed at 24 and 36 hour timepoints. In another specific embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce the expression, activity, serum concentration, and/or release of mast cell leukotrienes, such as C4, D4, and E4 by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art. In another specific embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce the expression, activity, serum concentration, and/or release of mast cell cytokines, such as TNF- $\alpha$ , IL-4, and IL-13 by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, an ELISA or Western blot assay).

**[00195]** In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce mast cell infiltration by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known in the art. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce mast cell proliferation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least

60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce mast cell infiltration by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vitro* and/or *in vivo* assay described herein or well-known in the art and inhibit and/or reduce mast cell proliferation at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In a preferred embodiment, reductions in mast cell infiltration may be measured *in vivo* by sensitizing animals to ovalbumin. Briefly, 100 µg of ovalbumin complexed with aluminum adjuvant is administered subcutaneously on days 1 and 21. Throughout the three-week sensitization procedure, animals are administered an IL-9 reactive antibody or a control antibody at a 10 mg/kg dose every 5 to 7 days. On days 29, 30 and 31, animals are exposed to ovalbumin without adjuvant by aerosol delivery, or alternatively, by intrasal instillation of 100 µl of a 1 µg/ml solution prepared in PBS. On day 31, 6 hours after the last ovalbumin challenge, animals are euthanized and lung tissue is fixed by perfusion with formalin. Mast cell infiltration is assessed histologically by counting mast cells per field in lung epithelial tissue sections. Using this experimental design, mast cell precursors may be differentiated from mast cells in lung epithelium by assessing (for example) whether metachromatic granules are present, and/or by immunohistochemistry using differentiation-dependent cell surface markers (e.g., FcεRI).

**[00196]** In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce infiltration of mast cell precursors in the upper and/or lower respiratory tracts by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known in the art. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce proliferation of mast cell precursors by at least 25%, at

least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, a trypan blue assay, FACS or  $^3\text{H}$  thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce infiltration of mast cell precursors into the upper and/or lower respiratory tracts by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known in the art and inhibit and/or reduce proliferation of mast cell precursors at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (*e.g.*, a trypan blue assay, FACS or  $^3\text{H}$  thymidine assay). In a preferred embodiment, mast cell precursor infiltration may be measured *in vivo* by the mast cell infiltration assay described *supra*.

**[00197]** In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide mediate depletion of peripheral blood T-cells by inducing an increase in apoptosis of T-cells, particularly Th2 cells. In a preferred embodiment, Th2 T lymphocyte depletion may be measured *in vivo* by sensitizing animals with ovalbumin. Briefly, 100  $\mu\text{g}$  of ovalbumin complexed with aluminum adjuvant is administered subcutaneously on days 1 and 21. Throughout the three-week sensitization procedure, animals are administered an IL-9 reactive antibody or a control antibody at a 10 mg/kg dose every 5 to 7 days. On day 28, animals receive a 100  $\mu\text{g}$  boost of ovalbumin protein without adjuvant intravenously. Two days following the intravenous boost, the animals are euthanized. Spleen cells are recovered and analyzed by flow cytometry. Splenic Th2 T lymphocytes, identifiable by cytoplasmic staining for IL-4, should be reduced in animals receiving an IL-9 neutralizing antibody compared with the control antibody recipients. In another embodiment, antibodies that immunospecifically bind to an IL-9 polypeptide mediate inhibit and/or reduce Th1 and Th2 differentiation by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to

one of skill in the art (e.g., FACS). In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce T cell infiltration, particularly Th2 cell infiltration, in the upper and/or lower respiratory tracts by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay well-known to one of skill in the art. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibits and/or reduce T cell proliferation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce T cell infiltration, particularly Th2 cell infiltration, in the upper and/or lower respiratory tracts by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%, inhibit and/or reduce T cell proliferation, particularly Th2 cell proliferation, by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%, and/or increases apoptosis of T cells relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art.

**[00198]** In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide reduce macrophage infiltration by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay well-known to one of skill in the art. In a preferred embodiment, reductions in macrophage infiltration may be measured *in vivo* by sensitizing animals to ovalbumin. Briefly, 100 µg of ovalbumin complexed with aluminum adjuvant is administered subcutaneously on days 1 and 21. Throughout the three-week sensitization procedure, animals are administered IL-9 reactive antibody or control antibody at 10 mg/kg dose every 5 to 7 days. On days 29, 30 and 31, animals are exposed to ovalbumin without adjuvant by aerosol delivery, or alternatively, by intrasal instillation of 100 µl of a 1 µg/ml solution prepared in PBS. On

day 31, 6 hours after the last ovalbumin challenge, animals are euthanized and lung tissue is fixed by perfusion with formalin. Macrophage infiltration is assessed by immunocytochemistry by counting CD14 positive cells per field in lung tissue sections. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce macrophage proliferation by at least 25%, preferably, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce macrophage infiltration by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art and inhibit and/or reduce macrophage proliferation at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art.

**[00199]** In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide reduce B cell infiltration by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art. In a preferred embodiment, reductions in B lymphocyte infiltration may be measured *in vivo* by systemically sensitizing animals to ovalbumin. Briefly, 100 µg of ovalbumin complexed with aluminum adjuvant is administered subcutaneously on days 1 and 21. Throughout the three-week sensitization procedure, animals are administered an IL-9 reactive antibody or a control antibody at a 10 mg/kg dose every 5 to 7 days. On days 29, 30 and 31, animals are exposed to ovalbumin without adjuvant by aerosol delivery, or alternatively, by intrasal instillation of 100 µl of a 1 µg/ml solution prepared in PBS. On day 31, 6 hours after the last ovalbumin challenge, animals are euthanized and lung tissue is fixed by perfusion with formalin. B lymphocyte infiltration is assessed by immunocytochemistry by counting CD19 positive cells per field

in lung tissue sections. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce B cell proliferation by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce B cell infiltration by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art and inhibits and/or reduces B cell proliferation at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art.

**[00200]** In certain embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide reduce eosinophil infiltration in the upper and/or lower respiratory tracts by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art (see, e.g., Li *et al.*, 2000, *Am. J. Respir. Cell Mol. Biol.* 25:644-51). In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce eosinophil proliferation, by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein (see Section 5.6) or well known to one of skill in the art (e.g., a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce eosinophil infiltration by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay



described herein or well-known to one of skill in the art and inhibits and/or reduces eosinophil proliferation at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art.

**[00201]** In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide reduce neutrophil infiltration by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well known to one of skill in the art. In other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce neutrophil proliferation, by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assays described herein or well-known to one of skill in the art (*e.g.*, a trypan blue assay, FACS or <sup>3</sup>H thymidine assay). In yet other embodiments, antibodies that immunospecifically bind to an IL-9 polypeptide inhibit and/or reduce neutrophil infiltration by at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art and inhibits and/or reduces neutrophil proliferation at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art.

**[00202]** In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide neutralizes or inhibits IL-9 mediated biological effects including, but not limited to inflammatory cell recruitment, epithelia hyperplasia, mucin production of epithelial cells, and mast cell activation, degranulation, proliferation, and/or infiltration.

**[00203]** In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide acts synergistically with a proteinaceous agent (*e.g.*, a peptide, polypeptide, or protein (including an antibody)) and/or a non-proteinaceous agent that

antagonizes the expression, function, and/or activity of IgE to reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells by at least 25%, preferably, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assays described herein or well known to one of skill in the art.

**[00204]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide acts synergistically with a proteinaceous agent (*e.g.*, a peptide, polypeptide, protein (including an antibody)) and/or a non-proteinaceous agent that antagonizes the expression, function, and/or activity of a mast cell protease to reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art.

**[00205]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide acts synergistically with a proteinaceous agent (*e.g.*, a peptide, polypeptide, and protein (including an antibody)) or a non-proteinaceous agent that antagonizes the expression, function, and/or activity of a stem cell factor to reduce or inhibit to reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells by at least 25%, preferably at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% relative to a control such as PBS or a control IgG antibody in an *in vivo* and/or *in vitro* assay described herein or well-known to one of skill in the art. In a preferred embodiment, primary mast cells or a mast cell line is cultured *in vitro* in the presence of 1 ng/ml IL-9 plus 1 ng/ml stem cell factor. Baseline levels of protease (*e.g.*, chymase and tryptase) and leukotriene are determined in the supernatant by commercially available ELISA kits. The ability of antibodies to modulate protease or leukotriene levels is assessed by adding IL-9 reactive antibody or control antibody directly to cell cultures at a concentration of 1 µg/ml. Protease and leukotriene levels are assessed at 24 and 36 hour time points.

**[00206]** The antibodies of the present invention that immunospecifically bind to an IL-9 polypeptide may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of an IL-9 polypeptide or

may be specific for both an IL-9 polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, International publications WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt, *et al.*, J. Immunol. 147:60-69(1991); U.S. Patent Nos. 4,474,893, 4,714,681, 4,925,648, 5,573,920, and 5,601,819; and Kostelny *et al.*, J. Immunol. 148:1547-1553 (1992).

**[00207]** The present invention provides for antibodies that have a high binding affinity for an IL-9 polypeptide. In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has an association rate constant or  $k_{on}$  rate (antibody (Ab) + antigen (Ag)  $\xrightarrow{k_{on}}$  Ab-Ag) of at least  $10^5 M^{-1}s^{-1}$ , at least  $1.5 \times 10^5 M^{-1}s^{-1}$ , at least  $2 \times 10^5 M^{-1}s^{-1}$ , at least  $2.5 \times 10^5 M^{-1}s^{-1}$ , at least  $5 \times 10^5 M^{-1}s^{-1}$ , at least  $10^6 M^{-1}s^{-1}$ , at least  $5 \times 10^6 M^{-1}s^{-1}$ , at least  $10^7 M^{-1}s^{-1}$ , at least  $5 \times 10^7 M^{-1}s^{-1}$ , or at least  $10^8 M^{-1}s^{-1}$ , or  $10^5 - 10^8 M^{-1}s^{-1}$ ,  $1.5 \times 10^5 M^{-1}s^{-1} - 1 \times 10^7 M^{-1}s^{-1}$ ,  $2 \times 10^5 - 1 \times 10^6 M^{-1}s^{-1}$ , or  $4.5 \times 10^5 \times 10^7 M^{-1}s^{-1}$ . In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $k_{on}$  of at least  $2 \times 10^5 M^{-1}s^{-1}$ , at least  $2.5 \times 10^5 M^{-1}s^{-1}$ , at least  $5 \times 10^5 M^{-1}s^{-1}$ , at least  $10^6 M^{-1}s^{-1}$ , at least  $5 \times 10^6 M^{-1}s^{-1}$ , at least  $10^7 M^{-1}s^{-1}$ , at least  $5 \times 10^7 M^{-1}s^{-1}$ , or at least  $10^8 M^{-1}s^{-1}$  as determined by a BIAcore assay and the antibody neutralizes human IL-9 in the microneutralization assay as described herein. In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $k_{on}$  of at most  $10^8 M^{-1}s^{-1}$ , at most  $10^9 M^{-1}s^{-1}$ , at most  $10^{10} M^{-1}s^{-1}$ , at most  $10^{11} M^{-1}s^{-1}$ , or at most  $10^{12} M^{-1}s^{-1}$  as determined by a BIAcore assay and the antibody neutralizes human IL-9 in the microneutralization assay as described herein. In accordance with these embodiments, such antibodies may comprise a VH domain and/or a VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4 or a VH CDR and/or a VL CDR of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4.

**[00208]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $k_{off}$  rate (antibody (Ab) + antigen (Ag)  $\xleftarrow{k_{off}}$  Ab-Ag) of less than  $10^{-3} s^{-1}$ , less than  $5 \times 10^{-3} s^{-1}$ , less than  $10^{-4} s^{-1}$ , less than  $2 \times 10^{-4} s^{-1}$ , less than  $5 \times 10^{-4} s^{-1}$ , less than  $10^{-5} s^{-1}$ , less than  $5 \times 10^{-5} s^{-1}$ , less than  $10^{-6} s^{-1}$ , less than  $5 \times 10^{-6} s^{-1}$ , less than  $10^{-7} s^{-1}$ , less than  $5 \times 10^{-7} s^{-1}$ , less than  $10^{-8} s^{-1}$ , less than  $5 \times 10^{-8} s^{-1}$ , less than  $10^{-9} s^{-1}$ , less than  $5 \times 10^{-9} s^{-1}$ , or less than  $10^{-10} s^{-1}$ , or  $10^{-3} - 10^{-10} s^{-1}$ ,  $10^{-4} - 10^{-8} s^{-1}$ , or  $10^{-5} - 10^{-8} s^{-1}$ . In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $k_{off}$  of  $10^{-5} s^{-1}$ , less than  $5 \times 10^{-5} s^{-1}$ , less than  $10^{-6} s^{-1}$ , less than  $5 \times 10^{-6} s^{-1}$ , less than  $10^{-7} s^{-1}$ , less than  $5 \times 10^{-7} s^{-1}$ , less than  $10^{-8} s^{-1}$ , less than  $5 \times 10^{-8} s^{-1}$ , less than  $10^{-9} s^{-1}$ , less than  $5 \times 10^{-9} s^{-1}$ , or less than  $10^{-10} s^{-1}$  as determined by a BIAcore assay and the antibody neutralizes

human IL-9 in the microneutralization assay described herein. In another preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $k_{off}$  of greater than  $10^{-13} s^{-1}$ , greater than  $10^{-12} s^{-1}$ , greater than  $10^{-11} s^{-1}$ , greater than  $10^{-10} s^{-1}$ , greater than  $10^{-9} s^{-1}$ , or greater than  $10^{-8} s^{-1}$ . In accordance with these embodiments, such antibodies may comprise a VH domain and/or a VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, or a VH CDR and/or a VL CDR of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4.

**[00209]** In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has an affinity constant or  $K_a$  ( $k_{on}/k_{off}$ ) of at least  $10^2 M^{-1}$ , at least  $5 \times 10^2 M^{-1}$ , at least  $10^3 M^{-1}$ , at least  $5 \times 10^3 M^{-1}$ , at least  $10^4 M^{-1}$ , at least  $5 \times 10^4 M^{-1}$ , at least  $10^5 M^{-1}$ , at least  $5 \times 10^5 M^{-1}$ , at least  $10^6 M^{-1}$ , at least  $5 \times 10^6 M^{-1}$ , at least  $10^7 M^{-1}$ , at least  $5 \times 10^7 M^{-1}$ , at least  $10^8 M^{-1}$ , at least  $5 \times 10^8 M^{-1}$ , at least  $10^9 M^{-1}$ , at least  $5 \times 10^9 M^{-1}$ , at least  $10^{10} M^{-1}$ , at least  $5 \times 10^{10} M^{-1}$ , at least  $10^{11} M^{-1}$ , at least  $5 \times 10^{11} M^{-1}$ , at least  $10^{12} M^{-1}$ , at least  $5 \times 10^{12} M^{-1}$ , at least  $10^{13} M^{-1}$ , at least  $5 \times 10^{13} M^{-1}$ , at least  $10^{14} M^{-1}$ , at least  $5 \times 10^{14} M^{-1}$ , at least  $10^{15} M^{-1}$ , or at least  $5 \times 10^{15} M^{-1}$ , or  $10^2 - 5 \times 10^5 M^{-1}$ ,  $10^4 - 1 \times 10^{10} M^{-1}$ , or  $10^5 - 1 \times 10^8 M^{-1}$ . In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $K_a$  of at most  $10^{11} M^{-1}$ , at most  $5 \times 10^{11} M^{-1}$ , at most  $10^{12} M^{-1}$ , at most  $5 \times 10^{12} M^{-1}$ , at most  $10^{13} M^{-1}$ , at most  $5 \times 10^{13} M^{-1}$ , at most  $10^{14} M^{-1}$ , or at most  $5 \times 10^{14} M^{-1}$ . In another embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a dissociation constant or  $K_d$  ( $k_{off}/k_{on}$ ) of less than  $10^{-5} M$ , less than  $5 \times 10^{-5} M$ , less than  $10^{-6} M$ , less than  $5 \times 10^{-6} M$ , less than  $10^{-7} M$ , less than  $5 \times 10^{-7} M$ , less than  $10^{-8} M$ , less than  $5 \times 10^{-8} M$ , less than  $10^{-9} M$ , less than  $5 \times 10^{-9} M$ , less than  $10^{-10} M$ , less than  $5 \times 10^{-10} M$ , less than  $10^{-11} M$ , less than  $5 \times 10^{-11} M$ , less than  $10^{-12} M$ , less than  $5 \times 10^{-12} M$ , less than  $10^{-13} M$ , less than  $5 \times 10^{-13} M$ , less than  $10^{-14} M$ , less than  $5 \times 10^{-14} M$ , less than  $10^{-15} M$ , or less than  $5 \times 10^{-15} M$  or  $10^{-2} M - 5 \times 10^{-5} M$ ,  $10^{-6} - 10^{-15} M$ , or  $10^{-8} - 10^{-14} M$ . In a preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $K_d$  of less than  $10^{-9} M$ , less than  $5 \times 10^{-9} M$ , less than  $10^{-10} M$ , less than  $5 \times 10^{-10} M$ , less than  $1 \times 10^{-11} M$ , less than  $5 \times 10^{-11} M$ , less than  $1 \times 10^{-12} M$ , less than  $5 \times 10^{-12} M$ , less than  $10^{-13} M$ , less than  $5 \times 10^{-13} M$  or less than  $1 \times 10^{-14} M$ , or  $10^{-9} M - 10^{-14} M$  as determined by a BIAcore assay and the antibody neutralizes human IL-9 in the microneutralization assay described herein. In another preferred embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide has a  $K_d$  of greater than  $10^{-9} M$ , greater than  $5 \times 10^{-9} M$ , greater than  $10^{-10} M$ , greater than  $5 \times 10^{-10} M$ , greater than  $10^{-11} M$ , greater than  $5 \times 10^{-11} M$ , greater than  $10^{-12} M$ , greater than  $5 \times 10^{-12} M$ , greater

than  $6 \times 10^{-12}$  M, greater than  $10^{-13}$  M, greater than  $5 \times 10^{-13}$  M, greater than  $10^{-14}$  M, greater than  $5 \times 10^{-14}$  M or greater than  $10^{-9}$  M -  $10^{-14}$  M. In accordance with these embodiments, such antibodies may comprise a VH domain and/or a VL domain of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4, or a VH CDR and/or a VL CDR of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4.

**[00210]** In certain embodiments, the antibodies of the invention do not include antibodies known in the art that immunospecifically bind to an IL-9 polypeptide. Non-limiting examples of known antibodies that immunospecifically bind to an IL-9 polypeptide include 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 7F3 22D3, 7F3com-2H2, 7F3com-3H5 or 7F3com-3D4.

**[00211]** In specific embodiments, antibodies of the invention bind antigenic epitope-bearing peptides and polypeptides of IL-9, and said antigenic epitope-bearing peptides and polypeptides comprise or consist of an amino acid sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50 contiguous amino acid residues, and, preferably, between about 15 to about 30 contiguous amino acids of IL-9 found in any species. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 8, at least 10, at least 15, at least 20, at least 25, at least at least 30, or at least 35 amino acid residues in length.

**[00212]** IL-9 epitope-bearing peptides, polypeptides, and fragments thereof may be produced by any conventional means. See, *e.g.*, Houghten, R. A. (1985) "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids," *Proc. Natl. Acad. Sci. USA* 82:5 13 1-5 135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U. S. Patent No. 4,631,211 to Houghten *et al.* (1986).

**[00213]** The present invention provides peptides, polypeptides and/or proteins comprising one or more variable or hypervariable regions of the antibodies described herein. Preferably, peptides, polypeptides or proteins comprising one or more variable or hypervariable regions of antibodies of the invention further comprise a heterologous amino acid sequence. In certain embodiments, such a heterologous amino acid sequence comprises at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 30 contiguous amino acid

residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 75 contiguous amino acid residues, at least 100 contiguous amino acid residues or more contiguous amino acid residues. Such peptides, polypeptides and/or proteins may be referred to as fusion proteins.

**[00214]** In a specific embodiment, peptides, polypeptides or proteins comprising one or more variable or hypervariable regions of the antibodies of the invention are 10 amino acid residues, 15 amino acid residues, 20 amino acid residues, 25 amino acid residues, 30 amino acid residues, 35 amino acid residues, 40 amino acid residues, 45 amino acid residues, 50 amino acid residues, 75 amino acid residues, 100 amino acid residues, 125 amino acid residues, 150 amino acid residues or more amino acid residues in length. In certain embodiments, peptides, polypeptides, or proteins comprising one or more variable or hypervariable regions of an antibody of the invention immunospecifically bind to an IL-9 polypeptide. In other embodiments, peptides, polypeptides, or proteins comprising one or more variable or hypervariable regions of an antibody of the invention do not immunospecifically bind to an IL-9 polypeptide.

**[00215]** In a specific embodiment, the present invention provides peptides, polypeptides and/or proteins comprising a VH domain and/or VL domain of one of the antibodies described herein (see Table 1, *supra*). In a preferred embodiment, the present invention provides peptides, polypeptides and/or proteins comprising one or more CDRs having the amino acid sequence of any of the CDRs listed in Table 1, *supra*. In accordance with these embodiments, the peptides, polypeptides or proteins may further comprise a heterologous amino acid sequence.

**[00216]** Peptides, polypeptides or proteins comprising one or more variable or hypervariable regions have utility, *e.g.*, in the production of anti-idiotypic antibodies which in turn may be used to prevent, treat, and/or ameliorate one or more symptoms associated with a disease or disorder (*e.g.*, an autoimmune disorder, an inflammatory disorder, a proliferative disorder or an infection (preferably, a respiratory infection)). The anti-idiotypic antibodies produced can also be utilized in immunoassays, such as, *e.g.*, ELISAs, for the detection of antibodies which comprise a variable or hypervariable region contained in the peptide, polypeptide or protein used in the production of the anti-idiotypic antibodies.

#### **5.1.1 Antibodies Having Increased Half-Lives**

**[00217]** The present invention provides for antibodies that immunospecifically bind to an IL-9 polypeptide which have an extended half-life *in vivo*. In particular, the present invention provides antibodies that immunospecifically bind to an IL-9 polypeptide which

have a half-life in a subject, preferably a mammal and most preferably a human, of greater than 3 days, greater than 7 days, greater than 10 days, preferably greater than 15 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months.

**[00218]** To prolong the serum circulation of antibodies (*e.g.*, monoclonal antibodies, single chain antibodies and Fab fragments) *in vivo*, for example, inert polymer molecules such as high molecular weight polyethyleneglycol (PEG) can be attached to the antibodies with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C-terminus of the antibodies or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation can be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by size-exclusion or by ion-exchange chromatography. PEG-derivatized antibodies can be tested for binding activity as well as for *in vivo* efficacy using methods well-known to those of skill in the art, for example, by immunoassays described herein.

**[00219]** Antibodies having an increased half-life *in vivo* can also be generated introducing one or more amino acid modifications (*i.e.*, substitutions, insertions or deletions) into an IgG constant domain, or FcRn binding fragment thereof (preferably a Fc or hinge-Fc domain fragment). See, *e.g.*, International Publication No. WO 98/23289; International Publication No. WO 97/34631; International Publication No. WO 02/060919; and U.S. Patent No. 6,277,375, each of which is incorporated herein by reference in its entirety.

**[00220]** Further, antibodies can be conjugated to albumin in order to make the antibody or antibody fragment more stable *in vivo* or have a longer half life *in vivo*. The techniques are well-known in the art, see, *e.g.*, International Publication Nos. WO 93/15199, WO 93/15200, and WO 01/77137; and European Patent No. EP 413,622, all of which are incorporated herein by reference.

### **5.1.2 Antibody Conjugates**

**[00221]** The present invention provides of antibodies or fragments thereof that immunospecifically binds to an IL-9 polypeptide recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least 10, at

least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids) to generate fusion proteins. In particular, the invention provides fusion proteins comprising an antigen-binding fragment of an antibody described herein (*e.g.*, a Fab fragment, Fd fragment, Fv fragment, F(ab)<sub>2</sub> fragment, a VH domain, a VH CDR, a VL domain or a VL CDR) and a heterologous protein, polypeptide, or peptide. Preferably, the heterologous protein, polypeptide, or peptide that the antibody or antibody fragment is fused to is useful for targeting the antibody to respiratory epithelial cells, mast cells, neutrophils, eosinophils, B cells, macrophages, or activated T cells. For example, an antibody that immunospecifically binds to a cell surface receptor expressed by a particular cell type (*e.g.*, a respiratory epithelial cell, a mast cell, a neutrophil, an eosinophil, a B cell, a macrophage, or an activated T cell) may be fused or conjugated to an antibody or fragment of the invention. In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide is fused or conjugated to an anti-stem cell factor or an anti-kit ligand. Methods for fusing or conjugating proteins, polypeptides, or peptides to an antibody or an antibody fragment are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, and 5,112,946; European Patent Nos. EP 307,434 and EP 367,166; International Publication Nos. WO 96/04388 and WO 91/06570; Ashkenazi *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88: 10535-10539; Zheng *et al.*, 1995, J. Immunol. 154:5590-5600; and Vil *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:11337-11341 (said references are incorporated herein by reference in their entireties).

**[00222]** Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of antibodies of the invention or fragments thereof (*e.g.*, antibodies or fragments thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458; Patten *et al.*, 1997, Curr. Opinion Biotechnol. 8:724-33; Harayama, 1998, Trends Biotechnol. 16(2):76-82; Hansson, *et al.*, 1999, J. Mol. Biol. 287:265-76; and Lorenzo and Blasco, 1998, Biotechniques 24(2):308-313 (each of these patents and publications are hereby incorporated by reference in its entirety). Antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding an antibody or fragment thereof that immunospecifically binds to an IL-9 polypeptide may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.



**[00223]** Moreover, the antibodies or fragments thereof can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin (“HA”) tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, 1984, Cell 37:767), and the “flag” tag.

**[00224]** In other embodiments, antibodies of the present invention or fragments thereof conjugated to a diagnostic or detectable agent. Such antibodies can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder (*e.g.*, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, or an infection (preferably, a respiratory infection)) as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials, such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ , and  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115}\text{In}$ ,  $^{113}\text{In}$ ,  $^{112}\text{In}$ , and  $^{111}\text{In}$ ), technetium ( $^{99}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ , and  $^{117}\text{Sn}$ ; and positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions.

**[00225]** The present invention further encompasses uses of antibodies or fragments thereof conjugated to a therapeutic moiety. An antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Therapeutic moieties include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine,

cytarabine, 5-fluorouracil decarbazine); alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP), and cisplatin); anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin); antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)); Auristatin molecules (*e.g.*, auristatin PHE, bryostatin 1, and solastatin 10; *see* Woyke *et al.*, *Antimicrob. Agents Chemother.* 46:3802-8 (2002), Woyke *et al.*, *Antimicrob. Agents Chemother.* 45:3580-4 (2001), Mohammad *et al.*, *Anticancer Drugs* 12:735-40 (2001), Wall *et al.*, *Biochem. Biophys. Res. Commun.* 266:76-80 (1999), Mohammad *et al.*, *Int. J. Oncol.* 15:367-72 (1999), all of which are incorporated herein by reference); hormones (*e.g.*, glucocorticoids, progestins, androgens, and estrogens), DNA-repair enzyme inhibitors (*e.g.*, etoposide or topotecan), kinase inhibitors (*e.g.*, compound ST1571, imatinib mesylate (Kantarjian *et al.*, *Clin Cancer Res.* 8(7):2167-76 (2002)); cytotoxic agents (*e.g.*, paclitaxel, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof and those compounds disclosed in U.S. Pat. Nos. 6,245,759, 6,399,633, 6,383,790, 6,335,156, 6,271,242, 6,242,196, 6,218,410, 6,218,372, 6,057,300, 6,034,053, 5,985,877, 5,958,769, 5,925,376, 5,922,844, 5,911,995, 5,872,223, 5,863,904, 5,840,745, 5,728,868, 5,648,239, 5,587,459); farnesyl transferase inhibitors (*e.g.*, R115777, BMS-214662, and those disclosed by, for example, U.S. Patent Nos: 6,458,935, 6,451,812, 6,440,974, 6,436,960, 6,432,959, 6,420,387, 6,414,145, 6,410,541, 6,410,539, 6,403,581, 6,399,615, 6,387,905, 6,372,747, 6,369,034, 6,362,188, 6,342,765, 6,342,487, 6,300,501, 6,268,363, 6,265,422, 6,248,756, 6,239,140, 6,232,338, 6,228,865, 6,228,856, 6,225,322, 6,218,406, 6,211,193, 6,187,786, 6,169,096, 6,159,984, 6,143,766, 6,133,303, 6,127,366, 6,124,465, 6,124,295, 6,103,723, 6,093,737, 6,090,948, 6,080,870, 6,077,853, 6,071,935, 6,066,738, 6,063,930, 6,054,466, 6,051,582, 6,051,574, and 6,040,305); topoisomerase inhibitors (*e.g.*, camptothecin; irinotecan; SN-38; topotecan; 9-aminocamptothecin; GG-211 (GI 147211); DX-8951f; IST-622; rubitecan; pyrazoloacridine; XR-5000; saintopin; UCE6; UCE1022; TAN-1518A; TAN-1518B; KT6006; KT6528; ED-110; NB-506; ED-110; NB-506; and rebeccamycin); bulgarein; DNA minor groove binders such as Hoescht dye 33342 and Hoechst dye 33258; nitidine; fagaronine; epiberberine; coralyne; beta-lapachone; BC-4-1; bisphosphonates (*e.g.*, alendronate, cimadronate, clodronate, tiludronate, etidronate, ibandronate, neridronate,

olpandronate, risedronate, piridronate, pamidronate, zoledronate) HMG-CoA reductase inhibitors, (*e.g.*, lovastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, statin, cerivastatin, lescol, lupitor, rosuvastatin and atorvastatin); antisense oligonucleotides (*e.g.*, those disclosed in the U.S. Pat. Nos. 6,277,832, 5,998,596, 5,885,834, 5,734,033, and 5,618,709); adenosine deaminase inhibitors (*e.g.*, Fludarabine phosphate and 2-Chlorodeoxyadenosine); ibritumomab tiuxetan (Zevalin®); tositumomab (Bexxar®) and pharmaceutically acceptable salts, solvates, clathrates, and prodrugs thereof.

**[00226]** Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety or drug moiety that modifies a given biological response. Therapeutic moieties or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein, peptide, or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF- $\alpha$ , TNF- $\beta$ , AIM I (see, International Publication No. WO 97/33899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, 1994, J. Immunol., 6:1567-1574), and VEGF (see, International Publication No. WO 99/23105), an anti-angiogenic agent, *e.g.*, angiostatin, endostatin or a component of the coagulation pathway (*e.g.*, tissue factor); or, a biological response modifier such as, for example, a lymphokine (*e.g.*, interferon gamma ("IFN- $\gamma$ "), interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-5 ("IL-5"), interleukin-6 ("IL-6"), interleukin-7 ("IL-7"), interleukin-10 ("IL-10"), interleukin-12 ("IL-12"), interleukin-15 ("IL-15"), interleukin-23 ("IL-23"), granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone ("GH")), or a coagulation agent (*e.g.*, calcium, vitamin K, tissue factors, such as but not limited to, Hageman factor (factor XII), high-molecular-weight kininogen (HMWK), prekallikrein (PK), coagulation proteins-factors II (prothrombin), factor V, XIIa, VIII, XIIIa, XI, XIa, IX, IXa, X, phospholipid, fibrinopeptides A and B from the  $\alpha$  and  $\beta$  chains of fibrinogen, fibrin monomer). In a specific embodiment, an antibody that immunospecifically binds to an IL-9 polypeptide is conjugated with a leukotriene antagonist (*e.g.*, montelukast, zafirlukast, pranlukast, and zileuton).

**[00227]** Moreover, an antibody can be conjugated to therapeutic moieties such as a radioactive metal ion, such as alpha-emitters such as  $^{213}\text{Bi}$  or macrocyclic chelators useful for conjugating radiometal ions, including but not limited to,  $^{131}\text{In}$ ,  $^{131}\text{L}$ ,  $^{131}\text{Y}$ ,  $^{131}\text{Ho}$ ,  $^{131}\text{Sm}$ , to polypeptides or any of those listed *supra*. In certain embodiments, the macrocyclic chelator

is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, 1998, Clin Cancer Res. 4(10):2483-90; Peterson *et al.*, 1999, Bioconjug. Chem. 10(4):553-7; and Zimmerman *et al.*, 1999, Nucl. Med. Biol. 26(8):943-50, each incorporated by reference in their entireties.

**[00228]** Techniques for conjugating therapeutic moieties to antibodies are well known, see, *e.g.*, Arnon *et al.*, “Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy”, in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, “Antibodies For Drug Delivery”, in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, “Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review”, in Monoclonal Antibodies 84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); “Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy”, in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, 1982, Immunol. Rev. 62:119-58.

**[00229]** Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

**[00230]** The therapeutic moiety or drug conjugated to an antibody that immunospecifically binds to an IL-9 polypeptide or fragment thereof should be chosen to achieve the desired prophylactic or therapeutic effect(s) for a particular disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, diseases or disorders associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, autoimmune disease, inflammatory disease, proliferative disease, or infection (preferably, a respiratory infection) in a subject. A clinician or other medical personnel should consider the following when deciding on which therapeutic moiety or drug to conjugate to an antibody that immunospecifically binds to an IL-9 polypeptide or fragment thereof: the nature of the disease, the severity of the disease, and the condition of the subject.

**[00231]** Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

## 5.2 THERAPIES USEFUL IN COMBINATION WITH IL-9 ANTIBODIES

[00232] The present invention also provides methods for preventing, managing, treating, and/or ameliorating diseases and disorders including, but not limited to, disorders characterized by aberrant expression and/or activity IL-9, disorders characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, inflammatory disorders, autoimmune disorders, proliferative disorders, or infections (preferably, respiratory infections) comprising administering to a subject in need thereof an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide and one or more therapies (*e.g.*, prophylactic or therapeutic agents) other than antibodies that immunospecifically bind to an IL-9 polypeptide (*see* U.S. Provisional Appn. No. 60/477,801, filed June 10, 2003, entitled “Methods of Preventing or Treating Respiratory Conditions,” U.S. Provisional Appn. No. 60/462,307, filed April 11, 2003, entitled “Methods of Preventing or Treating Respiratory Conditions,” and a U.S. Provisional Application (identified by Attorney Docket No. 10271-113-999) filed concurrently herewith (April 12, 2004), entitled “Methods of Preventing or Treating Respiratory Conditions,” which are all incorporated by reference herein in their entireties). The present invention also provides compositions comprising one or more antibodies that immunospecifically bind to an IL-9 polypeptide and one or more prophylactic or therapeutic agents other than antibodies that immunospecifically bind to an IL-9 peptide and methods of preventing, managing, treating, and/or ameliorating a disease or disorder utilizing said compositions. Therapeutic or prophylactic agents include, but are not limited to, small molecules, synthetic drugs, peptides, polypeptides, proteins, nucleic acids (*e.g.*, DNA and RNA nucleotides including, but not limited to, antisense nucleotide sequences, triple helices, RNAi, and nucleotide sequences encoding biologically active proteins, polypeptides or peptides) antibodies, synthetic or natural inorganic molecules, mimetic agents, and synthetic or natural organic molecules.

[00233] Any therapy (*e.g.*, prophylactic or therapeutic agents) which is known to be useful, or which has been used or is currently being used for the prevention, management, treatment, or amelioration of one or more symptoms associated with a disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disorder or disease associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder or an infection (preferably, a respiratory infection) can be used in combination with an antibody that immunospecifically binds to an IL-9 polypeptide in accordance with the invention described herein. See, *e.g.*, Gilman *et al.*, *Goodman and Gilman's: The*

*Pharmacological Basis of Therapeutics*, Tenth Ed., McGraw-Hill, New York, 2001; The *Merck Manual of Diagnosis and Therapy*, Berkow, M.D. *et al.* (eds.), 17th Ed., Merck Sharp & Dohme Research Laboratories, Rahway, NJ, 1999; and *Cecil Textbook of Medicine*, 20th Ed., Bennett and Plum (eds.), W.B. Saunders, Philadelphia, 1996 for information regarding therapies, in particular prophylactic or therapeutic agents, which have been or are currently being used for preventing, treating, managing, and/or ameliorating disorders associated with aberrant expression and/or activity of an IL-9 polypeptide, disorders associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, autoimmune disorders, inflammatory disorders, proliferative disorders or infections. Examples of prophylactic and therapeutic agents include, but are not limited to, immunomodulatory agents, anti-inflammatory agents (*e.g.*, adrenocorticoids, corticosteroids (*e.g.*, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone), glucocorticoids, steroids, non-steroidal anti-inflammatory drugs (*e.g.*, aspirin, ibuprofen, diclofenac, and COX-2 inhibitors), and leukotriene antagonists (*e.g.*, montelukast, methyl xanthines, zafirlukast, and zileuton), beta2-agonists (*e.g.*, albuterol, biterol, fenoterol, isoetharine, metaproterenol, pirbuterol, salbutamol, terbutaline, formoterol, salmeterol, and salbutamol terbutaline), anticholinergic agents (*e.g.*, ipratropium bromide and oxitropium bromide), sulphasalazine, penicillamine, dapsone, antihistamines, anti-malarial agents (*e.g.*, hydroxychloroquine), anti-viral agents, and antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, erythromycin, penicillin, mithramycin, and anthramycin (AMC)).

### **5.2.1 Immunomodulatory Therapies**

[00234] Any immunomodulatory agent well-known to one of skill in the art may be used in the methods and compositions of the invention. Immunomodulatory agents can affect one or more or all aspects of the immune response in a subject. Aspects of the immune response include, but are not limited to, the inflammatory response, the complement cascade, leukocyte and lymphocyte differentiation, proliferation, and/or effector function, monocyte and/or basophil counts, and the cellular communication among cells of the immune system. In certain embodiments of the invention, an immunomodulatory agent modulates one aspect of the immune response. In other embodiments, an immunomodulatory agent modulates more than one aspect of the immune response. In a preferred embodiment of the invention, the administration of an immunomodulatory agent to a subject inhibits or reduces one or more aspects of the subject's immune response capabilities. In a specific embodiment of the invention, the

immunomodulatory agent inhibits or suppresses the immune response in a subject. In accordance with the invention, an immunomodulatory agent is not antibody that immunospecifically binds to an IL-9 polypeptide. In certain embodiments, an immunomodulatory agent is not an anti-inflammatory agent. In certain embodiments, an immunomodulatory agent is not an anti-angiogenic agent. In other embodiments, an immunomodulatory agent is not an integrin  $\alpha_v\beta_3$  antagonist. In other embodiments, an immunomodulatory agent is not a TNF  $\alpha$  antagonist. In certain embodiments, an immunomodulatory agent is a chemotherapeutic agent. In certain embodiments, an immunomodulatory agent is not a chemotherapeutic agent.

**[00235]** Examples of immunomodulatory agents include, but are not limited to, proteinaceous agents such as cytokines, peptide mimetics, and antibodies (*e.g.*, human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab or F(ab)<sub>2</sub> fragments or epitope binding fragments), nucleic acid molecules (*e.g.*, antisense nucleic acid molecules and triple helices), small molecules, organic compounds, and inorganic compounds. In particular, immunomodulatory agents include, but are not limited to, methotrexate, leflunomide, cyclophosphamide, cytoxan, Immuran, cyclosporine A, minocycline, azathioprine, antibiotics (*e.g.*, FK506 (tacrolimus)), methylprednisolone (MP), corticosteroids, steroids, mycophenolate mofetil, rapamycin (sirolimus), mizoribine, deoxyspergualin, brequinar, malononitriloamides (*e.g.*, leflunamide), T cell receptor modulators, cytokine receptor modulators, and modulators mast cell modulators.

**[00236]** For clarification regarding T cell receptor modulators, cytokine receptor modulators, and mast cell modulators see Section 3.1. Examples of T cell receptor modulators include, but are not limited to, anti-T cell receptor antibodies (*e.g.*, anti-CD4 antibodies (*e.g.*, cM-T412 (Boeringer), IDEC-CE9.1® (IDEC and SKB), mAB 4162W94, Orthoclone and OKTcdr4a (Janssen-Cilag)), anti-CD3 antibodies (*e.g.*, Nuvion (Product Design Labs), OKT3 (Johnson & Johnson), or Rituxan (IDEC)), anti-CD5 antibodies (*e.g.*, an anti-CD5 ricin-linked immunoconjugate), anti-CD7 antibodies (*e.g.*, CHH-380 (Novartis)), anti-CD8 antibodies, anti-CD40 ligand monoclonal antibodies (*e.g.*, IDEC-131 (IDEC)), anti-CD52 antibodies (*e.g.*, CAMPATH 1H (Ilex)), anti-CD2 antibodies (*e.g.*, sipilizumab (MedImmune, Inc., International Publication Nos. WO 02/098370 and WO 02/069904)), anti-CD11a antibodies (*e.g.*, Xanelim (Genentech)), and anti-B7 antibodies (*e.g.*, IDEC-114 (IDEC))), CTLA4-immunoglobulin, and LFA-3TIP (Biogen, International Publication No. WO 93/08656 and U.S. Patent No. 6,162,432).

**[00237]** Examples of cytokine receptor modulators include, but are not limited to, soluble cytokine receptors (*e.g.*, the extracellular domain of a TNF- $\alpha$  receptor or a fragment

thereof, the extracellular domain of an IL-1 $\beta$  receptor or a fragment thereof, and the extracellular domain of an IL-6 receptor or a fragment thereof), cytokines or fragments thereof (*e.g.*, interleukin IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-23, TNF- $\alpha$ , TNF- $\beta$ , interferon (IFN)- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , and GM-CSF), anti-cytokine receptor antibodies (*e.g.*, anti-IFN receptor antibodies, anti-IL-2 receptor antibodies (*e.g.*, Zenapax (Protein Design Labs)), anti-IL-3 receptor antibodies, anti-IL-4 receptor antibodies, anti-IL-6 receptor antibodies, anti-IL-10 receptor antibodies, anti-IL-12 receptor antibodies, anti-IL-13 receptor antibodies, anti-IL-15 receptor antibodies, and anti-IL-23 receptor antibodies), anti-cytokine antibodies (*e.g.*, anti-IFN antibodies, anti-TNF- $\alpha$  antibodies, anti-IL-1 $\beta$  antibodies, anti-IL-3 antibodies, anti-IL-6 antibodies, anti-IL-8 antibodies (*e.g.*, ABX-IL-8 (Abgenix)), anti-IL-12 antibodies, anti-IL-13 antibodies, anti-IL-15 antibodies, and anti-IL-23 antibodies).

**[00238]** In a specific embodiment, a cytokine receptor modulator is IL-3, IL-4, IL-10, or a fragment thereof. In another embodiment, a cytokine receptor modulator is an anti-IL-1 $\beta$  antibody, anti-IL-6 antibody, anti-IL-12 receptor antibody, or anti-TNF- $\alpha$  antibody. In another embodiment, a cytokine receptor modulator is the extracellular domain of a TNF- $\alpha$  receptor or a fragment thereof. In certain embodiments, a cytokine receptor modulator is not a TNF- $\alpha$  antagonist.

**[00239]** In one embodiment, a cytokine receptor modulator is a mast cell modulator. In an alternative embodiment, a cytokine receptor modulator is not a mast cell modulator. Examples of mast cell modulators include, but are not limited to stem cell factor (c-kit receptor ligand) inhibitors (*e.g.*, mAb 7H6, mAb 8H7a, pAb 1337, FK506, CsA, dexamthasone, and fluconcinonide), c-kit receptor inhibitors (*e.g.*, STI 571 (formerly known as CGP 57148B)), mast cell protease inhibitors (*e.g.*, GW-45, GW-58, wortmannin, LY 294002, calphostin C, cytochalasin D, genistein, KT5926, staurosporine, and lactoferrin), relaxin ("RLX"), IgE antagonists (*e.g.*, antibodies rhuMAb-E25 omalizumab, HMK-12 and 6HD5, and mAB Hu-901), IL-3 antagonists, IL-4 antagonists, IL-10 antagonists, and TGF-beta.

**[00240]** An immunomodulatory agent may be selected to interfere with the interactions between the T helper subsets (TH1 or TH2) and B cells to inhibit neutralizing antibody formation. Antibodies that interfere with or block the interactions necessary for the activation of B cells by TH (T helper) cells, and thus block the production of neutralizing antibodies, are useful as immunomodulatory agents in the methods of the invention. For example, B cell activation by T cells requires certain interactions to occur (Durie *et al.*, Immunol. Today, 15(9):406-410 (1994)), such as the binding of CD40 ligand



on the T helper cell to the CD40 antigen on the B cell, and the binding of the CD28 and/or CTLA4 ligands on the T cell to the B7 antigen on the B cell. Without both interactions, the B cell cannot be activated to induce production of the neutralizing antibody.

**[00241]** The CD40 ligand (CD40L)-CD40 interaction is a desirable point to block the immune response because of its broad activity in both T helper cell activation and function as well as the absence of redundancy in its signaling pathway. Thus, in a specific embodiment of the invention, the interaction of CD40L with CD40 is transiently blocked at the time of administration of one or more of the immunomodulatory agents. This can be accomplished by treating with an agent which blocks the CD40 ligand on the TH cell and interferes with the normal binding of CD40 ligand on the T helper cell with the CD40 antigen on the B cell. An antibody to CD40 ligand (anti-CD40L) (available from Bristol-Myers Squibb Co; see, *e.g.*, European patent application 555,880, published Aug. 18, 1993) or a soluble CD40 molecule can be selected and used as an immunomodulatory agent in accordance with the methods of the invention.

**[00242]** An immunomodulatory agent may be selected to inhibit the interaction between TH1 cells and cytotoxic T lymphocytes ("CTLs") to reduce the occurrence of CTL-mediated killing. An immunomodulatory agent may be selected to alter (*e.g.*, inhibit or suppress) the proliferation, differentiation, activity and/or function of the CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells. For example, antibodies specific for T cells can be used as immunomodulatory agents to deplete, or alter the proliferation, differentiation, activity and/or function of CD4<sup>+</sup> and/or CD8<sup>+</sup> T cells.

**[00243]** In one embodiment of the invention, an immunomodulatory agent that reduces or depletes T cells, preferably memory T cells, is administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance with the methods of the invention. See, *e.g.*, U.S. Pat. No. 4,658,019. In another embodiment of the invention, an immunomodulatory agent that inactivates CD8<sup>+</sup> T cells is administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance

with the methods of the invention. In a specific embodiment, anti-CD8 antibodies are used to reduce or deplete CD8<sup>+</sup> T cells.

**[00244]** In another embodiment, an immunomodulatory agent which reduces or inhibits one or more biological activities (*e.g.*, the differentiation, proliferation, and/or effector functions) of TH0, TH1, and/or TH2 subsets of CD4<sup>+</sup> T helper cells is administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance with the methods of the invention. One example of such an immunomodulatory agent is IL-4. IL-4 enhances antigen-specific activity of TH2 cells at the expense of the TH1 cell function (see, *e.g.*, Yokota et al, 1986 Proc. Natl. Acad. Sci., USA, 83:5894-5898; and U.S. Pat. No. 5,017,691). Other examples of immunomodulatory agents that affect the biological activity (*e.g.*, proliferation, differentiation, and/or effector functions) of T-helper cells (in particular, TH1 and/or TH2 cells) include, but are not limited to, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-23, and interferon (IFN)- $\gamma$ .

**[00245]** In another embodiment, an immunomodulatory agent administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance with the methods of the invention is a cytokine that prevents antigen presentation. In a specific embodiment, an immunomodulatory agent used in the methods of the invention is IL-10. IL-10 also reduces or inhibits macrophage action which involves bacterial elimination.

**[00246]** An immunomodulatory agent may be selected to reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells. In certain embodiments, the immunomodulatory agent interferes with the interactions between mast cells and mast cell activating agents, including, but not limited to stem cell factors (c-kit ligands), IgE, IL-4, environmental irritants, and infectious agents. In a specific embodiment, the immunomodulatory agent reduces or inhibits the response of mast cells to environmental irritants such as, but not limited to pollen, dust mites, tobacco smoke, and/or pet dander. In another specific embodiment, the immunomodulatory agent reduces or inhibits the response of mast cells to infectious agents, such as viruses, bacteria, and fungi.

Examples of mast cell modulators that reduce or inhibit the activation, degranulation, proliferation, and/or infiltration of mast cells include, but are not limited to, stem cell factor (c-kit receptor ligand) inhibitors (*e.g.*, mAb 7H6, mAb 8H7a, and pAb 1337 (see Mendiaz *et al.*, 1996, *Eur J Biochem* 293(3):842-849), FK506 and CsA (Ito *et al.*, 1999 *Arch Dermatol Res* 291(5):275-283), dexamthasone and fluconcinonide (see Finooto *et al.* *J Clin Invest* 1997 99(7):1721-1728)), c-kit receptor inhibitors (*e.g.*, STI 571 (formerly known as CGP 57148B) (see Heinrich *et al.*, 2000 *Blood* 96(3):925-932)), mast cell protease inhibitors (*e.g.*, GW-45 and GW-58 (see Temkin *et al.*, 2002 *J Immunol* 169(5):2662-2669), wortmannin, LY 294002, calphostin C, and cytochalasin D (see Vosseller *et al.*, 1997, *Mol Biol Cell* 1997:909-922), genistein, KT5926, and staurosporine (see Nagai *et al.*, 1995, *Biochem Biophys Res Commun* 208(2):576-581), and lactoferrin (see He *et al.*, 2003 *Biochem Pharmacol* 65(6):1007-1015)), relaxin ("RLX") (see Bani *et al.*, 2002 *Int Immunopharmacol* 2(8):1195-1294), ), IgE antagonists (*e.g.*, antibodies rhuMAB-E25 omalizumab (see Finn *et al.*, 2003 *J Allergy Clin Immuno* 111(2):278-284; Corren *et al.*, 2003 *J Allergy Clin Immuno* 111(1):87-90; Busse and Neaville, 2001 *Curr Opin Allergy Clin Immuno* 1(1):105-108; and Tang and Powell, 2001, *Eur J Pediatr* 160(12): 696-704), HMK-12 and 6HD5 (see Miyajima *et al.*, 2002 *Int Arch Allergy Immuno* 128(1):24-32), and mAB Hu-901 (see van Neerven *et al.*, 2001 *Int Arch Allergy Immuno* 124(1-3):400), IL-3 antagonist, IL-4 antagonists, IL-10 antagonists, and TGF-beta (see Metcalfe *et al.*, 1995, *Exp Dermatol* 4(4 Pt 2):227-230).

**[00247]** In a preferred embodiment, proteins, polypeptides or peptides (including antibodies) that are utilized as immunomodulatory agents are derived from the same species as the recipient of the proteins, polypeptides or peptides so as to reduce the likelihood of an immune response to those proteins, polypeptides or peptides. In another preferred embodiment, when the subject is a human, the proteins, polypeptides, or peptides that are utilized as immunomodulatory agents are human or humanized.

**[00248]** In accordance with the invention, one or more immunomodulatory agents are administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) prior to, subsequent to, or concomitantly with an antibody that immunospecifically binds to an IL-9 polypeptide. Preferably, one or more immunomodulatory agents are administered in combination with an antibody that immunospecifically binds to an IL-9 polypeptide to a subject at risk of or with a disease or

disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) to reduce or inhibit one or more aspects of the immune response as deemed necessary by one of skill in the art. Any technique well-known to one skilled in the art can be used to measure one or more aspects of the immune response in a particular subject, and thereby determine when it is necessary to administer an immunomodulatory agent to said subject. In a preferred embodiment, a mean absolute lymphocyte count of approximately 500 cells/mm<sup>3</sup>, preferably 600 cells/mm<sup>3</sup>, 650 cells/mm<sup>3</sup>, 700 cells/mm<sup>3</sup>, 750 cells/mm<sup>3</sup>, 800 cells/mm<sup>3</sup>, 900 cells/mm<sup>3</sup>, 1000 cells/mm<sup>3</sup>, 1100 cells/mm<sup>3</sup>, or 1200 cells/mm<sup>3</sup> is maintained in a subject. In another preferred embodiment, a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) is not administered an immunomodulatory agent if their absolute lymphocyte count is 500 cells/mm<sup>3</sup> or less, 550 cells/mm<sup>3</sup> or less, 600 cells/mm<sup>3</sup> or less, 650 cells/mm<sup>3</sup> or less, 700 cells/mm<sup>3</sup> or less, 750 cells/mm<sup>3</sup> or less, or 800 cells/mm<sup>3</sup> or less.

**[00249]** In a preferred embodiment, one or more immunomodulatory agents are administered in combination with an antibody that immunospecifically binds to an IL-9 polypeptide to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) so as to transiently reduce or inhibit one or more aspects of the immune response. Such a transient inhibition or reduction of one or more aspects of the immune system can last for hours, days, weeks, or months. Preferably, the transient inhibition or reduction in one or more aspects of the immune response lasts for a few hours (*e.g.*, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 14 hours, 16 hours, 18 hours, 24 hours, 36 hours, or 48 hours), a few days (*e.g.*, 3 days, 4 days, 5 days, 6 days, 7 days, or 14 days), or a few weeks (*e.g.*, 3 weeks, 4 weeks, 5 weeks or 6 weeks). The transient reduction or inhibition of one or more aspects of the immune response enhances the prophylactic and/or therapeutic effect(s) of an antibody that immunospecifically binds to an IL-9 polypeptide.

**[00250]** Nucleic acid molecules encoding proteins, polypeptides, or peptides with immunomodulatory activity or proteins, polypeptides, or peptides with immunomodulatory activity can be administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance with the methods of the invention. Further, nucleic acid molecules encoding derivatives, analogs, or fragments of proteins, polypeptides, or peptides with immunomodulatory activity, or derivatives, analogs, or fragments of proteins, polypeptides, or peptides with immunomodulatory activity can be administered to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) in accordance with the methods of the invention. Preferably, such derivatives, analogs, and fragments retain the immunomodulatory activity of the full-length, wild-type protein, polypeptide, or peptide.

**[00251]** Preferably, agents that are commercially available and known to function as immunomodulatory agents are used in the methods of the invention. The immunomodulatory activity of an agent can be determined *in vitro* and/or *in vivo* by any technique well-known to one skilled in the art, including, *e.g.*, by CTL assays, proliferation assays, and immunoassays (*e.g.* ELISAs) for the expression of particular proteins such as co-stimulatory molecules and cytokines.

### **5.2.2 Anti-angiogenic Therapies**

**[00252]** Any anti-angiogenic agent well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-angiogenic agents include proteins, polypeptides, peptides, fusion proteins, antibodies (*e.g.*, human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab fragments, F(ab)<sub>2</sub> fragments, and antigen-binding fragments thereof) such as antibodies that immunospecifically bind to TNF- $\alpha$ , nucleic acid molecules (*e.g.*, antisense molecules or triple helices), organic molecules, inorganic molecules, and small molecules that reduce or inhibit angiogenesis. In particular, examples of anti-angiogenic agents, include, but are not limited to, endostatin, angiostatin, apomigren, anti-angiogenic antithrombin III, the 29 kDa

N-terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, a uPA receptor antagonist, the 16 kDa proteolytic fragment of prolactin, the 7.8 kDa proteolytic fragment of platelet factor-4, the anti-angiogenic 24 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, RGD and NGR containing peptides, the small anti-angiogenic peptides of laminin, fibronectin, procollagen and EGF, integrin  $\alpha_v\beta_3$  antagonists, acid fibroblast growth factor (aFGF) antagonists, basic fibroblast growth factor (bFGF) antagonists, vascular endothelial growth factor (VEGF) antagonists (*e.g.*, anti-VEGF antibodies), and VEGF receptor (VEGFR) antagonists (*e.g.*, anti-VEGFR antibodies).

**[00253]** Examples of integrin  $\alpha_v\beta_3$  antagonists include, but are not limited to, proteinaceous agents such as non-catalytic metalloproteinase fragments, RGD peptides, peptide mimetics, fusion proteins, disintegrins or derivatives or analogs thereof, and antibodies that immunospecifically bind to integrin  $\alpha_v\beta_3$ , nucleic acid molecules, organic molecules, and inorganic molecules. Non-limiting examples of antibodies that immunospecifically bind to integrin  $\alpha_v\beta_3$  include 11D2 (Searle), LM609 (Scripps), and VITAXIN™ (MedImmune, Inc.). Non-limiting examples of small molecule peptidometric integrin  $\alpha_v\beta_3$  antagonists include S836 (Searle) and S448 (Searle). Examples of disintegrins include, but are not limited to, Accutin. The invention also encompasses the use of any of the integrin  $\alpha_v\beta_3$  antagonists disclosed in the following U.S. Patents and International publications in the compositions and methods of the invention: U.S. Patent Nos. 5,149,780; 5,196,511; 5,204,445; 5,262,520; 5,306,620; 5,478,725; 5,498,694; 5,523,209; 5,578,704; 5,589,570; 5,652,109; 5,652,110; 5,693,612; 5,705,481; 5,753,230; 5,767,071; 5,770,565; 5,780,426; 5,817,457; 5,830,678; 5,849,692; 5,955,572; 5,985,278; 6,048,861; 6,090,944; 6,096,707; 6,130,231; 6,153,628; 6,160,099; and 6,171,588; and International Publication Nos. WO 95/22543; WO 98/33919; WO 00/78815; and WO 02/070007, each of which is incorporated herein by reference in its entirety. In a preferred embodiment, the anti-angiogenic agent is VITAXIN™ (MedImmune, Inc.) or an antigen-binding fragment thereof.

**[00254]** In a specific embodiment of the invention, an anti-angiogenic agent is endostatin. Naturally occurring endostatin consists of the C-terminal ~180 amino acids of collagen XVIII (cDNAs encoding two splice forms of collagen XVIII have GenBank Accession Nos. AF18081 and AF18082). In another embodiment of the invention, an anti-angiogenic agent is a plasminogen fragment (the coding sequence for plasminogen can be found in GenBank Accession Nos. NM\_000301 and A33096). Angiostatin peptides

naturally include the four kringle domains of plasminogen, kringle 1 through kringle 4. It has been demonstrated that recombinant kringle 1, 2 and 3 possess the anti-angiogenic properties of the native peptide, whereas kringle 4 has no such activity (Cao *et al.*, 1996, J. Biol. Chem. 271:29461-29467). Accordingly, the angiostatin peptides comprises at least one and preferably more than one kringle domain selected from the group consisting of kringle 1, kringle 2 and kringle 3. In a specific embodiment, the anti-angiogenic peptide is the 40 kDa isoform of the human angiostatin molecule, the 42 kDa isoform of the human angiostatin molecule, the 45 kDa isoform of the human angiostatin molecule, or a combination thereof. In another embodiment, an anti-angiogenic agent is the kringle 5 domain of plasminogen, which is a more potent inhibitor of angiogenesis than angiostatin (angiostatin comprises kringle domains 1-4). In another embodiment of the invention, an anti-angiogenic agent is antithrombin III. Antithrombin III, which is referred to hereinafter as antithrombin, comprises a heparin binding domain that tethers the protein to the vasculature walls, and an active site loop which interacts with thrombin. When antithrombin is tethered to heparin, the protein elicits a conformational change that allows the active loop to interact with thrombin, resulting in the proteolytic cleavage of said loop by thrombin. The proteolytic cleavage event results in another change of conformation of antithrombin, which (i) alters the interaction interface between thrombin and antithrombin and (ii) releases the complex from heparin (Carrell, 1999, Science 285:1861-1862, and references therein). O'Reilly *et al.* (1999, Science 285:1926-1928) have discovered that the cleaved antithrombin has potent anti-angiogenic activity. Accordingly, in one embodiment, an anti-angiogenic agent is the anti-angiogenic form of antithrombin. In another embodiment of the invention, an anti-angiogenic agent is the 40 kDa and/or 29 kDa proteolytic fragment of fibronectin.

**[00255]** In another embodiment of the invention, an anti-angiogenic agent is a urokinase plasminogen activator (uPA) receptor antagonist. In one mode of the embodiment, the antagonist is a dominant negative mutant of uPA (see, *e.g.*, Crowley *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:5021-5025). In another mode of the embodiment, the antagonist is a peptide antagonist or a fusion protein thereof (Goodson *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:7129-7133). In yet another mode of the embodiment, the antagonist is a dominant negative soluble uPA receptor (Min *et al.*, 1996, Cancer Res. 56:2428-2433). In another embodiment of the invention, a therapeutic molecule of the invention is the 16 kDa N-terminal fragment of prolactin, comprising approximately 120 amino acids, or a biologically active fragment thereof (the coding sequence for prolactin can be found in GenBank Accession No. NM\_000948). In another embodiment of the

invention, an anti-angiogenic agent is the 7.8 kDa platelet factor-4 fragment. In another embodiment of the invention, a therapeutic molecule of the invention is a small peptide corresponding to the anti-angiogenic 13 amino acid fragment of platelet factor-4, the anti-angiogenic factor designated 13.40, the anti-angiogenic 22 amino acid peptide fragment of thrombospondin I, the anti-angiogenic 20 amino acid peptide fragment of SPARC, the small anti-angiogenic peptides of laminin, fibronectin, procollagen, or EGF, or small peptide antagonists of integrin  $\alpha_v\beta_3$  or the VEGF receptor. In another embodiment, the small peptide comprises an RGD or NGR motif. In certain embodiments, an anti-angiogenic agent is a TNF- $\alpha$  antagonist. In other embodiments, an anti-angiogenic agent is not a TNF- $\alpha$  antagonist.

**[00256]** Nucleic acid molecules encoding proteins, polypeptides, or peptides with anti-angiogenic activity, or proteins, polypeptides or peptides with anti-angiogenic activity can be administered to a subject at risk of or with a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection in accordance with the methods of the invention. Further, nucleic acid molecules encoding derivatives, analogs, fragments, or variants of proteins, polypeptides, or peptides with anti-angiogenic activity, or derivatives, analogs, fragments, or variants of proteins, polypeptides, or peptides with anti-angiogenic activity can be administered to a subject at risk of or with a disease or disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection in accordance with the methods of the invention. Preferably, such derivatives, analogs, variants, and fragments retain the anti-angiogenic activity of the full-length, wild-type protein, polypeptide, or peptide.

**[00257]** Proteins, polypeptides, or peptides that can be used as anti-angiogenic agents can be produced by any technique well-known in the art or described herein. Proteins, polypeptides or peptides with anti-angiogenic activity can be engineered so as to increase the *in vivo* half-life of such proteins, polypeptides, or peptides utilizing techniques well-known in the art or described herein. Preferably, anti-angiogenic agents that are commercially available are used in the compositions and methods of the invention. The anti-angiogenic activity of an agent can be determined *in vitro* and/or *in vivo* by any technique well-known to one skilled in the art.



### 5.2.3 TNF- $\alpha$ Antagonists

**[00258]** Any TNF- $\alpha$  antagonist well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of TNF- $\alpha$  antagonists include proteins, polypeptides, peptides, fusion proteins, antibodies (*e.g.*, human, humanized, chimeric, monoclonal, polyclonal, Fvs, ScFvs, Fab fragments, F(ab)<sub>2</sub> fragments, and antigen-binding fragments thereof) such as antibodies that immunospecifically bind to TNF- $\alpha$ , nucleic acid molecules (*e.g.*, antisense molecules or triple helices), organic molecules, inorganic molecules, and small molecules that blocks, reduces, inhibits or neutralizes a function, an activity and/or expression of TNF- $\alpha$ . In various embodiments, a TNF- $\alpha$  antagonist reduces the function, activity and/or expression of TNF- $\alpha$  by at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% relative to a control such as phosphate buffered saline (PBS).

**[00259]** Examples of antibodies that immunospecifically bind to TNF- $\alpha$  include, but are not limited to, infliximab (REMICADE®; Centacor), D2E7 (Abbott Laboratories/Knoll Pharmaceuticals Co., Mt. Olive, N.J.), CDP571 which is also known as HUMICADE™ and CDP-870 (both of Celltech/Pharmacia, Slough, U.K.), and TN3-19.12 (Williams *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91: 2762-2766; Thorbecke *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:7375-7379). The present invention also encompasses the use of antibodies that immunospecifically bind to TNF- $\alpha$  disclosed in the following U.S. Patents in the compositions and methods of the invention: 5,136,021; 5,147,638; 5,223,395; 5,231,024; 5,334,380; 5,360,716; 5,426,181; 5,436,154; 5,610,279; 5,644,034; 5,656,272; 5,658,746; 5,698,195; 5,736,138; 5,741,488; 5,808,029; 5,919,452; 5,958,412; 5,959,087; 5,968,741; 5,994,510; 6,036,978; 6,114,517; and 6,171,787; each of which are herein incorporated by reference in their entirety. Examples of soluble TNF- $\alpha$  receptors include, but are not limited to, sTNF-R1 (Amgen), etanercept (ENBRELEL™; Immunex) and its rat homolog RENBRELEL™, soluble inhibitors of TNF- $\alpha$  derived from TNFRI, TNFRII (Kohn *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:8331-8335), and TNF- $\alpha$  Inh (Seckinger *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:5188-5192).

**[00260]** In one embodiment, a TNF- $\alpha$  antagonist used in the compositions and methods of the invention is a soluble TNF- $\alpha$  receptor. In a specific embodiment, a TNF- $\alpha$  antagonist used in the compositions and methods of the invention is etanercept (ENBRELEL™; Immunex) or a fragment, derivative or analog thereof. In another embodiment, a TNF- $\alpha$  antagonist used in the compositions and methods of the invention is

an antibody that immunospecifically binds to TNF- $\alpha$ . In a specific embodiment, a TNF- $\alpha$  antagonist used in the compositions and methods of the invention is infliximab (REMICADE®; Centacor) a derivative, analog or antigen-binding fragment thereof.

**[00261]** Other TNF- $\alpha$  antagonists encompassed by the invention include, but are not limited to, IL-10, which is known to block TNF- $\alpha$  production via interferon  $\gamma$ -activated macrophages (Oswald *et al.* 1992, Proc. Natl. Acad. Sci. USA 89:8676-8680), TNFR-IgG (Ashkenazi *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:10535-10539), the murine product TBP-1 (Serono/Yeda), the vaccine CytoTAB (Protherics), antisense molecule 104838 (ISIS), the peptide RDP-58 (SangStat), thalidomide (Celgene), CDC-801 (Celgene), DPC-333 (Dupont), VX-745 (Vertex), AGIX-4207 (AtheroGenics), ITF-2357 (Italfarmaco), NPI-13021-31 (Nereus), SCIO-469 (Scios), TACE targeter (Immunix/AHP), CLX-120500 (Calyx), Thiazolopyrim (Dynavax), auranofin (Ridaura) (SmithKline Beecham Pharmaceuticals), quinacrine (mepacrine dichlorohydrate), tenidap (Enablex), Melanin (Large Scale Biological), and anti-p38 MAPK agents by Uriach.

**[00262]** Nucleic acid molecules encoding proteins, polypeptides, or peptides with TNF- $\alpha$  antagonist activity, or proteins, polypeptides, or peptides with TNF- $\alpha$  antagonist activity can be administered to a subject at risk of or with an inflammatory or autoimmune disease in accordance with the methods of the invention. Further, nucleic acid molecules encoding derivatives, analogs, fragments or variants of proteins, polypeptides, or peptides with TNF- $\alpha$  antagonist activity, or derivatives, analogs, fragments or variants of proteins, polypeptides, or peptides with TNF- $\alpha$  antagonist activity can be administered to a subject at risk of or with an inflammatory or autoimmune disease in accordance with the methods of the invention. Preferably, such derivatives, analogs, variants and fragments retain the TNF- $\alpha$  antagonist activity of the full-length, wild-type protein, polypeptide, or peptide.

**[00263]** Proteins, polypeptides, or peptides that can be used as TNF- $\alpha$  antagonists can be produced by any technique well-known in the art or described herein. Proteins, polypeptides or peptides with TNF- $\alpha$  antagonist activity can be engineered so as to increase the *in vivo* half-life of such proteins, polypeptides, or peptides utilizing techniques well-known in the art or described herein. Preferably, agents that are commercially available and known to function as TNF- $\alpha$  antagonists are used in the compositions and methods of the invention. The TNF- $\alpha$  antagonist activity of an agent can be determined *in vitro* and/or *in vivo* by any technique well-known to one skilled in the art.

#### 5.2.4 Anti-inflammatory Therapies

[00264] Any anti-inflammatory agent, including agents useful in therapies for inflammatory disorders, well-known to one of skill in the art can be used in the compositions and methods of the invention. Non-limiting examples of anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs, anticholinergics (*e.g.*, atropine sulfate, atropine methylnitrate, and ipratropium bromide (ATROVENT™)), beta2-agonists (*e.g.*, albuterol (VENTOLIN™ and PROVENTIL™), bitolterol (TORNALATE™), levalbuterol (XOPONEX™), metaproterenol (ALUPENT™), pirbuterol (MAXAIR™), terbutaline (BRETHAIRE™ and BRETHINE™), albuterol (PROVENTIL™, REPETABS™, and VOLMAX™), formoterol (FORADIL AEROLIZER™), and salmeterol (SEREVENT™ and SEREVENT DISKUS™)), and methylxanthines (*e.g.*, theophylline (UNIPHYL™, THEO-DUR™, SLO-BID™, AND TEHO-42™)). Examples of NSAIDs include, but are not limited to, aspirin, ibuprofen, celecoxib (CELEBREX™), diclofenac (VOLTAREN™), etodolac (LODINE™), fenoprofen (NALFON™), indomethacin (INDOCIN™), ketoralac (TORADOL™), oxaprozin (DAYPRO™), nabumentone (RELAFENT™), sulindac (CLINORIL™), tolmetin (TOLECTIN™), rofecoxib (VIOXX™), naproxen (ALEVE™, NAPROSYN™), ketoprofen (ACTRON™) and nabumetone (RELAFENT™). Such NSAIDs function by inhibiting a cyclooxygenase enzyme (*e.g.*, COX-1 and/or COX-2). Examples of steroidal anti-inflammatory drugs include, but are not limited to, glucocorticoids, dexamethasone (DECADRON™), corticosteroids (*e.g.*, methylprednisolone (MEDROL™)), cortisone, hydrocortisone, prednisone (PREDNISON™ and DELTASONE™), prednisolone (PRELONE™ and PEDIAPRED™), triamcinolone, azulfidine, and inhibitors of eicosanoids (*e.g.*, prostaglandins, thromboxanes, and leukotrienes (*see* Table 2, *infra*, for non-limiting examples of leukotriene and typical dosages of such agents)).

[00265] In certain embodiments, the anti-inflammatory agent is an agent useful in the prevention, management, treatment, and/or amelioration of asthma or one or more symptoms thereof. Non-limiting examples of such agents include adrenergic stimulants (*e.g.*, catecholamines (*e.g.*, epinephrine, isoproterenol, and isoetharine), resorcinols (*e.g.*, metaproterenol, terbutaline, and fenoterol), and saligenins (*e.g.*, salbutamol)), adrenocorticoids, glucocorticoids, corticosteroids (*e.g.*, beclomethadonse, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, and prednisone), other steroids, beta2-agonists (*e.g.*, albuterol, bitolterol, fenoterol, isoetharine, metaproterenol, pirbuterol, salbutamol, terbutaline, formoterol, salmeterol, and albutamol

terbutaline), anti-cholinergics (*e.g.*, ipratropium bromide and oxitropium bromide), IL-4 antagonists (including antibodies), IL-5 antagonists (including antibodies), IL-13 antagonists (including antibodies), PDE4-inhibitor, NF-Kappa- $\beta$  inhibitor, VLA-4 inhibitor, CpG, anti-CD23, selectin antagonists (TBC 1269), mast cell protease inhibitors (*e.g.*, tryptase kinase inhibitors (*e.g.*, GW-45, GW-58, and genisteine), phosphatidylinositide-3' (PI3)-kinase inhibitors (*e.g.*, calphostin C), and other kinase inhibitors (*e.g.*, staurosporine) (see Temkin *et al.*, 2002 J Immunol 169(5):2662-2669; Vosseller *et al.*, 1997 Mol. Biol. Cell 8(5):909-922; and Nagai *et al.*, 1995 Biochem Biophys Res Commun 208(2):576-581)), a C3 receptor antagonists (including antibodies), immunosuppressant agents (*e.g.*, methotrexate and gold salts), mast cell modulators (*e.g.*, cromolyn sodium (INTAL<sup>TM</sup>) and nedocromil sodium (TILADE<sup>TM</sup>)), and mucolytic agents (*e.g.*, acetylcysteine)). In a specific embodiment, the anti-inflammatory agent is a leukotriene inhibitor (*e.g.*, montelukast (SINGULAIR<sup>TM</sup>), zafirlukast (ACCOLATE<sup>TM</sup>), pranlukast (ONON<sup>TM</sup>), or zileuton (ZYFLO<sup>TM</sup>) (*see* Table 2)).

**Table 2. Leukotriene Inhibitors for Asthma Therapy**

Leukotriene Modifier	Usual Daily Dosage
Montelukast (SINGULAIR <sup>TM</sup> )	4 mg for 2-5 years old 5 mg for 6 to 15 years old 10mg for 15 years and older
Zafirlukast (ACCOLATE <sup>TM</sup> )	10 mg b.i.d. for 5 to 12 years old twice daily 20 mg b.i.d. for 12 years or older twice daily
Pranlukast (ONON <sup>TM</sup> )	Only available in Asia
Zileuton (ZYFLO <sup>TM</sup> )	600 mg four times a day for 12 years and older

[00266] In certain embodiments, the anti-inflammatory agent is an agent useful in preventing, treating, managing, and/or ameliorating allergies or one or more symptoms thereof. Non-limiting examples of such agents include antimediator drugs (*e.g.*, antihistamine, *see* Table 3, *infra* for non-limiting examples of antihistamine and typical dosages of such agents), corticosteroids, decongestants, sympathomimetic drugs (*e.g.*,  $\alpha$ -adrenergic and  $\beta$ -adrenergic drugs), TNX901 (Leung *et al.*, 2003, N Engl J Med 348(11):986-993), IgE antagonists (*e.g.*, antibodies rhuMAb-E25 omalizumab (see Finn *et al.*, 2003 J Allergy Clin Immuno 111(2):278-284; Corren *et al.*, 2003 J Allergy Clin Immuno 111(1):87-90; Busse and Neaville, 2001 Curr Opin Allergy Clin Immuno 1(1):105-108; and Tang and Powell, 2001, Eur J Pediatr 160(12): 696-704), HMK-12 and 6HD5 (see Miyajima *et al.*, 2002 Int Arch Allergy Immuno 128(1):24-32), and mAB Hu-901 (see van Neerven *et al.*, 2001 Int Arch Allergy Immuno 124(1-3):400), theophylline and its

derivatives, glucocorticoids, and immunotherapies (*e.g.*, repeated long-term injection of allergen, short course desensitization, and venom immunotherapy).

**Table 3. H<sub>1</sub> Antihistamines**

<b>Chemical class and representative drugs</b>	<b>Usual daily dosage</b>
Ethanolamine Diphenhydramine Clemastine	25-50 mg every 4-6 hours 0.34-2.68 mg every 12 hours
Ethylenediamine Tripeleminamine	25-50 mg every 4-6 hours
Alkylamine Brompheniramine  Chlorpheniramine  Triprolidine (1.25 mg/5ml)	4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour 4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour 2.5 mg every 4-6 hours
Phenothiazine Promethazine	25 mg at bedtime
Piperazine Hydroxyzine	25 mg every 6-8 hours
Piperidines Astemizole (nonsedating) Azatadine Cetirizine Cyproheptadine Fexofenadine (nonsedating) Loratidine (nonsedating)	10 mg/day 1-2 mg every 12 hours 10 mg/day 4 mg every 6-8 hour 60 mg every 12 hours 10 mg every 24 hours

[00267] Anti-inflammatory therapies and their dosages, routes of administration, and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

### **5.2.5 Anti-Cancer Therapies**

[00268] Any therapy (*e.g.*, therapeutic or prophylactic agent) which is known to be useful, has been used, or is currently being used for the prevention, treatment, management, or amelioration of a proliferative disorder, such as cancer, or one or more symptoms thereof can be used in compositions and method of the invention. Therapies (*e.g.*, therapeutic or prophylactic agents) include, but are not limited to, peptides, polypeptides, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, and organic molecules. Non-limiting examples of cancer therapies include

chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies.

**[00269]** In certain embodiments, the anti-cancer agent is an immunomodulatory agent, such as a chemotherapeutic agent. In certain other embodiments, the anti-cancer agent is an immunomodulatory agent other than a chemotherapeutic agent. In other embodiments, the anti-cancer agent is not an immunomodulatory agent. In specific embodiments, the anti-cancer agent is an anti-angiogenic agent. In other embodiments, the anti-cancer agent is not an anti-angiogenic agent. In specific embodiments, the anti-cancer agent is an anti-inflammatory agent. In other embodiments, the anti-cancer agent is not an anti-inflammatory agent.

**[00270]** In particular embodiments, the anti-cancer agent is, but not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bisphosphonates (*e.g.*, pamidronate (Aredria), sodium clodronate (Bonefos), zoledronic acid (Zometa), alendronate (Fosamax), etidronate, ibandronate, cimadronate, risedronate, and tiludronate); bizelesin; bleomycin sulfate; brequinar sodium; broprimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; EphA2 inhibitors (*e.g.*, anti-EphA2 antibodies that result in the phosphorylation of EphA2 and the degradation of EphA2 (see, U.S. Patent Application No. 60/418,213 which is incorporated herein by reference in its entirety); elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alpha-2a; interferon alpha-2b; interferon alpha-n1 ; interferon alpha-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide

acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; anti-CD2 antibodies (*e.g.*, sipilizumab (MedImmune Inc.; International Publication No. WO 02/098370, which is incorporated herein by reference in its entirety)); megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedapa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; pipsulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprime; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride.

**[00271]** Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D<sub>3</sub>; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine;

carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorIns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; daclicximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastrine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; HMG CoA reductase inhibitors (*e.g.*, atorvastatin, cerivastatin, fluvastatin, lescol, lupitor, lovastatin, rosuvastatin, and simvastatin); hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4- iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; LFA-3TIP (Biogen, Cambridge, MA; International Publication No. WO 93/0686 and U.S. Patent No. 6,162,432); liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocil; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal



antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safinol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; 5-fluorouracil; leucovorin; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin;

toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; thalidomide; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; VITAXIN™ (see U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents”); vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[00272] In specific embodiments, radiation therapy comprising the use of x-rays, gamma rays and other sources of radiation to destroy the cancer cells is used in combination with the antibodies of the invention. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy, wherein the radiation is directed from a remote source. In other preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass.

[00273] Cancer therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (56<sup>th</sup> ed., 2002).

### **5.2.6 Anti-Viral Agents**

[00274] Any anti-viral agent well-known to one of skill in the art can be used in the compositions and the methods of the invention. Non-limiting examples of anti-viral agents include proteins, polypeptides, peptides, fusion proteins antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce the attachment of a virus to its receptor, the internalization of a virus into a cell, the replication of a virus, or release of virus from a cell. In particular, anti-viral agents include, but are not limited to, nucleoside analogs (*e.g.*, zidovudine, acyclovir, gangcyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin), foscarnet, amantadine, rimantadine, saquinavir, indinavir, ritonavir, alpha-interferons and other interferons, and AZT.

[00275] In specific embodiments, the anti-viral agent is an immunomodulatory agent that is immunospecific for a viral antigen. As used herein, the term “viral antigen” includes, but is not limited to, any viral peptide, polypeptide and protein (*e.g.*, HIV gp120, HIV nef, RSV F glycoprotein, RSV G glycoprotein, influenza virus neuraminidase,

influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (*e.g.*, gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. Antibodies useful in this invention for treatment of a viral infectious disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: adenoviridae (*e.g.*, mastadenovirus and aviadenovirus), herpesviridae (*e.g.*, herpes simplex virus 1, herpes simplex virus 2, herpes simplex virus 5, and herpes simplex virus 6), leviviridae (*e.g.*, levivirus, enterobacteria phase MS2, allovirus), poxviridae (*e.g.*, chordopoxvirinae, parapoxvirus, avipoxvirus, capripoxvirus, leporipoxvirus, suipoxvirus, molluscipoxvirus, and entomopoxvirinae), papovaviridae (*e.g.*, polyomavirus and papillomavirus), paramyxoviridae (*e.g.*, paramyxovirus, parainfluenza virus 1, morbillivirus (*e.g.*, measles virus), rubulavirus (*e.g.*, mumps virus), pneumonovirinae (*e.g.*, pneumovirus, human respiratory syncytial virus), and metapneumovirus (*e.g.*, avian pneumovirus and human metapneumovirus)), picornaviridae (*e.g.*, enterovirus, rhinovirus, hepatovirus (*e.g.*, human hepatitis A virus), cardiovirus, and aphthovirus), reoviridae (*e.g.*, orthoreovirus, orbivirus, rotavirus, cypovirus, fijivirus, phytoreovirus, and oryzavirus), retroviridae (*e.g.*, mammalian type B retroviruses, mammalian type C retroviruses, avian type C retroviruses, type D retrovirus group, BLV-HTLV retroviruses, lentivirus (*e.g.* human immunodeficiency virus 1 and human immunodeficiency virus 2), spumavirus), flaviviridae (*e.g.*, hepatitis C virus), hepadnaviridae (*e.g.*, hepatitis B virus), togaviridae (*e.g.*, alphavirus (*e.g.*, sindbis virus) and rubivirus (*e.g.*, rubella virus)), rhabdoviridae (*e.g.*, vesiculovirus, lyssavirus, ephemerovirus, cytorhabdovirus, and nucleorhabdovirus), arenaviridae (*e.g.*, arenavirus, lymphocytic choriomeningitis virus, Lassa virus, and Lassa virus), and coronaviridae (*e.g.*, coronavirus and torovirus).

**[00276]** Specific examples of antibodies available useful for the treatment of a viral infectious disease include, but are not limited to, PRO542 (Progenics) which is a CD4 fusion antibody useful for the treatment of HIV infection; Ostavir (Protein Design Labs, Inc., CA) which is a human antibody useful for the treatment of hepatitis B virus; and Protovir (Protein Design Labs, Inc., CA) which is a humanized IgG1 antibody useful for the treatment of cytomegalovirus (CMV); and palivizumab (SYNAGIS®; MedImmune, Inc.; International Publication No. WO 02/43660) which is a humanized antibody useful for treatment of RSV.

**[00277]** In a specific embodiment, the anti-viral agents used in the compositions and methods of the invention inhibit or reduce a pulmonary or respiratory virus infection, inhibit or reduce the replication of a virus that causes a pulmonary or respiratory infection,

or inhibit or reduce the spread of a virus that causes a pulmonary or respiratory infection to other cells or subjects. In another preferred embodiment, the anti-viral agents used in the compositions and methods of the invention inhibit or reduce infection by RSV, hMPV, or PIV, inhibit or reduce the replication of RSV, hMPV, or PIV, or inhibit or reduce the spread of RSV, hMPV, or PIV to other cells or subjects. Examples of such agents and methods of treatment of RSV, hMPV, and/or PIV infections include, but are not limited to, nucleoside analogs, such as zidovudine, acyclovir, gangcyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin, as well as foscarnet, amantadine, rimantadine, saquinavir, indinavir, ritonavir, and the alpha-interferons. See U.S. Prov. Patent App. No. 60/398,475 filed July 25, 2002, entitled "Methods of Treating and Preventing RSV, HMPV, and PIV Using Anti-RSV, Anti-HMPV, and Anti-PIV Antibodies," and U.S. Patent App. No. 10/371,122 filed February 21, 2003, which are incorporated herein by reference in its entirety.

**[00278]** In preferred embodiments, the viral infection is RSV and the anti-viral antigen is an antibody that immunospecifically binds to an antigen of RSV. In certain embodiments, the anti-RSV-antigen antibody binds immunospecifically to an RSV antigen of the Group A of RSV. In other embodiments, the anti-RSV-antigen antibody binds immunospecifically to an RSV antigen of the Group B of RSV. In other embodiments, an antibody binds to an antigen of RSV of one Group and cross reacts with the analogous antigen of the other Group. In particular embodiments, the anti-RSV-antigen antibody binds immunospecifically to a RSV nucleoprotein, RSV phosphoprotein, RSV matrix protein, RSV small hydrophobic protein, RSV RNA-dependent RNA polymerase, RSV F protein, and/or RSV G protein. In additional specific embodiments, the anti-RSV-antigen antibody binds to allelic variants of a RSV nucleoprotein, a RSV nucleocapsid protein, a RSV phosphoprotein, a RSV matrix protein, a RSV attachment glycoprotein, a RSV fusion glycoprotein, a RSV nucleocapsid protein, a RSV matrix protein, a RSV small hydrophobic protein, a RSV RNA-dependent RNA polymerase, a RSV F protein, a RSV L protein, a RSV P protein, and/or a RSV G protein.

**[00279]** It should be recognized that antibodies that immunospecifically bind to a RSV antigen are known in the art. For example, palivizumab (SYNAGIS®) is a humanized monoclonal antibody presently used for the prevention of RSV infection in pediatric patients. In a specific embodiment, an antibody to be used with the methods of the present invention is palivizumab or an antibody-binding fragment thereof (*e.g.*, a fragment containing one or more complementarity determining regions (CDRs) and preferably, the variable domain of palivizumab). The amino acid sequence of palivizumab is disclosed, *e.g.*, in Johnson *et al.*, 1997, J. Infectious Disease 176:1215-1224, and U.S. Patent No.

5,824,307 and International Application Publication No.: WO 02/43660, entitled “Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment”, by Young *et al.*, which are incorporated herein by reference in their entireties.

**[00280]** One or more antibodies or antigen-binding fragments thereof that bind immunospecifically to a RSV antigen comprise a Fc domain with a higher affinity for the FcRn receptor than the Fc domain of palivizumab can also be used in accordance with the invention. Such antibodies are described in U.S. Pat. Appn. No. 10/020,354, filed December 12, 2001, which is incorporated herein by reference in its entireties. Further, one or more of the anti-RSV-antigen antibodies A4B4; P12f2 P12f4; P11d4; Ale9; A12a6; A13c4; A17d4; A4B4; 1X-493L1; FR H3-3F4; M3H9; Y10H6; DG; AFFF; AFFF(1); 6H8; L1-7E5; L2-15B10; A13a11; A1h5; A4B4(1); A4B4-F52S; or A4B4L1FR-S28R can be used in accordance with the invention. These antibodies are disclosed in International Application Publication No.: WO 02/43660, entitled “Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment”, by Young *et al.*, and US Provisional Patent Application 60/398,475 filed July 25, 2002, entitled “Methods of Treating and Preventing RSV, HMPV, and PIV Using Anti-RSV, Anti-HMPV, and Anti-PIV Antibodies” which are incorporated herein by reference in their entireties.

**[00281]** In certain embodiments, the anti-RSV-antigen antibodies are the anti-RSV-antigen antibodies of or are prepared by the methods of U.S. Application No: 09/724,531, filed November 28, 2000; 09/996,288, filed November 28, 2001; and U.S. Pat. Publication No. US2003/0091584 A1, published May 15, 2003, all entitled “Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment”, by Young *et al.*, which are incorporated by reference herein in their entireties. Methods and composition for stabilized antibody formulations that can be used in the methods of the present invention are disclosed in U.S. Provisional Application Nos. 60/388,921, filed June 14, 2002, and 60/388,920, filed June 14, 2002, which are incorporated by reference herein in their entireties.

**[00282]** Anti-viral therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (56<sup>th</sup> ed., 2002). Additional information on respiratory viral infections is available in *Cecil Textbook of Medicine* (18th ed., 1988).

### **5.2.7 Anti-Bacterial Agents**

**[00283]** Anti-bacterial agents and therapies well known to one of skill in the art for the prevention, treatment, management, or amelioration of bacterial infections can be used

in the compositions and methods of the invention. Non-limiting examples of anti-bacterial agents include proteins, polypeptides, peptides, fusion proteins, antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit or reduce a bacterial infection, inhibit or reduce the replication of bacteria, or inhibit or reduce the spread of bacteria to other subjects. In particular, examples of anti-bacterial agents include, but are not limited to, penicillin, cephalosporin, imipenem, aztreonam, vancomycin, cycloserine, bacitracin, chloramphenicol, erythromycin, clindamycin, tetracycline, streptomycin, tobramycin, gentamicin, amikacin, kanamycin, neomycin, spectinomycin, trimethoprim, norfloxacin, rifampin, polymyxin, amphotericin B, nystatin, ketocanazole, isoniazid, metronidazole, and pentamidine.

**[00284]** In a preferred embodiment, the anti-bacterial agent is an agent that inhibits or reduces a pulmonary or respiratory bacterial infection, inhibits or reduces the replication of a bacteria that causes a pulmonary or respiratory infection, or inhibits or reduces the spread of a bacteria that causes a pulmonary or respiratory infection to other subjects. In cases in which the pulmonary or respiratory bacterial infection is a mycoplasma infection (*e.g.*, pharyngitis, tracheobronchitis, and pneumonia), the anti-bacterial agent is preferably a tetracycline, erythromycin, or spectinomycin. In cases in which the pulmonary or respiratory bacterial infection is pneumonia caused by an aerobic gram negative bacilli (GNB), the anti-bacterial agent is preferably penicillin, first second, or third generation cephalosporin (*e.g.*, cefaclor, cefadroxil, cephalexin, or cephazolin), erythromycin, clindamycin, an aminoglycoside (*e.g.*, gentamicin, tobramycin, or amikacin), or a monolactam (*e.g.*, aztreonam). In cases in which the pulmonary or respiratory bacterial infection is tuberculosis, the anti-bacterial agent is preferably, rifampin, isoniazid, pyrazinamide, ethambutol, and streptomycin. In cases in which the respiratory infection is recurrent aspiration pneumonia, the anti-bacterial agent is preferably penicillin, an aminoglycoside, or a second or third generation cephalosporin.

**[00285]** Anti-bacterial therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (56<sup>th</sup> ed., 2002). Additional information on respiratory infections and anti-bacterial therapies is available in *Cecil Textbook of Medicine* (18th ed., 1988).

### **5.2.8 Anti-Fungal Agents**

**[00286]** Anti-fungal agents and therapies well known to one of skill in the art for prevention, management, treatment, and/or amelioration of a fungal infection or one or

more symptoms thereof (*e.g.*, a fungal respiratory infection) can be used in the compositions and methods of the invention. Non-limiting examples of anti-fungal agents include proteins, polypeptides, peptides, fusion proteins, antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce fungal infection, inhibit and/or reduce the replication of fungi, or inhibit and/or reduce the spread of fungi to other subjects. Specific examples of anti-fungal agents include, but are not limited to, azole drugs (*e.g.*, miconazole, ketoconazole (NIZORAL®), caspofungin acetate (CANCIDAS®), imidazole, triazoles (*e.g.*, fluconazole (DIFLUCAN®)), and itraconazole (SPORANOX®)), polyene (*e.g.*, nystatin, amphotericin B (FUNGIZONE®), amphotericin B lipid complex (“ABLC”)(ABELCET®), amphotericin B colloidal dispersion (“ABCD”)(AMPHOTEC®), liposomal amphotericin B (AMBISOME®)), potassium iodide (KI), pyrimidine (*e.g.*, flucytosine (ANCOBON®)), and voriconazole (VFEND®). See, *e.g.*, Table 4 for a list of specific anti-fungal agents and their recommended dosages.

**Table 4. Anti-fungal Agents**

Anti-fungal Agent	Dosage
Amphotericin B ABELCET® (lipid complex injection) AMBISOME® (liposome for injection) AMPHOTEC® (complex for injection)	5 mg/kg/day 3 - 5 mg/kg/day 3 - 4 mg/kg/day
Caspofungin acetate (CANCIDAS®)	70 mg on day one followed by 50 mg/day
Fluconazole (DIFLUCAN®)	up to 400 mg/day (adults) up to 12 mg/kg/day (children)
Itraconazole (SPORANOX®)	200 - 400 mg/day
Flucytosine (ANCOBON®)	50 - 150 mg/kg/day in divided dose every 6 hours
Liposomal nystatin	1 - 4 mg/kg
Ketoconazole (NIZORAL®)	200 mg single daily dose up to 400 mg/day in two divided doses (adults) 3.3 - 6.6 mg/kg/day for children 2 years old and older
Voriconazole (VFEND®)	6 mg/kg i.v. loading dose every 12 hours for two doses, followed by maintenance dose of 4 mg/kg i.v. every 12 hours, then oral maintenance dose of 200 - 100 mg tablet

[00287] In certain embodiments, the anti-fungal agent is an agent that inhibits or reduces a respiratory fungal infection, inhibits or reduces the replication of a fungus that

causes a pulmonary or respiratory infection, or inhibits or reduces the spread of a fungus that causes a pulmonary or respiratory infection to other subjects. In cases in which the pulmonary or respiratory fungal infection is *Blastomyces dermatitidis*, the anti-fungal agent is preferably itraconazole, amphotericin B, fluconazole, or ketoconazole. In cases in which the pulmonary or respiratory fungal infection is pulmonary aspergilloma, the anti-fungal agent is preferably amphotericin B, liposomal amphotericin B, itraconazole, or fluconazole. In cases in which the pulmonary or respiratory fungal infection is histoplasmosis, the anti-fungal agent is preferably amphotericin B, itraconazole, fluconazole, or ketoconazole. In cases in which the pulmonary or respiratory fungal infection is coccidioidomycosis, the anti-fungal agent is preferably fluconazole or amphotericin B. In cases in which the pulmonary or respiratory fungal infection is cryptococcosis, the anti-fungal agent is preferably amphotericin B, fluconazole, or combination of the two agents. In cases in which the pulmonary or respiratory fungal infection is chromomycosis, the anti-fungal agent is preferably itraconazole, fluconazole, or flucytosine. In cases in which the pulmonary or respiratory fungal infection is mucormycosis, the anti-fungal agent is preferably amphotericin B or liposomal amphotericin B. In cases in which the pulmonary or respiratory fungal infection is pseudoallescheriasis, the anti-fungal agent is preferably itraconazole or miconazole.

**[00288]** Anti-fungal therapies and their dosages, routes of administration, and recommended usage are known in the art and have been described in such literature as Dodds *et al.*, 2000 *Pharmacotherapy* 20(11) 1335-1355, the *Physician's Desk Reference* (57th ed., 2003) and the *Merk Manual of Diagnosis and Therapy* (17th ed., 1999).

### **5.3 PROPHYLACTIC & THERAPEUTIC USES OF ANTIBODIES**

**[00289]** The present invention is directed to therapies which involve administering one of more antibodies of the invention and compositions comprising said antibodies to a subject, preferably a human subject, for preventing, treating, managing, and/or ameliorating disease or disorder or one or more symptoms thereof. In one embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a disease or disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In certain embodiments, an effective amount of one or more polypeptides, peptides, and proteins comprising one or more antibodies or antibody fragments of the invention is administered to a subject in need thereof to prevent, treat, manage, and/or ameliorate a disease or disorder or one or more symptoms thereof.



**[00290]** The invention also provides methods of preventing, treating, managing, and/or ameliorating a disease or disorder or one or more symptoms thereof, said methods comprising administering to a subject in need thereof one or more of the antibodies of the invention and one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention that are currently being used, have been used, or are known to be useful in the prevention, treatment, management, and/or amelioration of said disease or disorder or one or more symptoms thereof. The prophylactic or therapeutic agents of the combination therapies of the invention can be administered sequentially or concurrently. In a specific embodiment, the combination therapies of the invention comprise an effective amount of one or more antibodies of the invention and an effective amount of at least one other therapy which has the same mechanism of action as said antibodies. In a specific embodiment, the combination therapies of the invention comprise an effective amount of one or more antibodies of the invention and an effective amount of at least one other therapy (*e.g.*, prophylactic or therapeutic agent) which has a different mechanism of action than said antibodies. In certain embodiments, the combination therapies of the present invention improve the prophylactic or therapeutic effect of one or more antibodies of the invention by functioning together with the antibodies to have an additive or synergistic effect. In certain embodiments, the combination therapies of the present invention reduce the side effects associated with the prophylactic or therapeutic agents.

**[00291]** The prophylactic or therapeutic agents of the combination therapies can be administered to a subject, preferably a human subject, in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration. *See* co-pending U.S. Provisional Appn. (identified by Attorney Docket Number 10271-126-888) filed concurrently herewith, entitled “Anti-IL-9 Antibody Formulations and Uses Thereof,” which is incorporated by reference herein in its entirety.

**[00292]** In a specific embodiment, a pharmaceutical composition comprising one or more antibodies of the invention described herein is administered to a subject, preferably a human, to prevent, treat, manage, and/or ameliorate a disease or disorder or one or more symptoms thereof. In accordance with the invention, pharmaceutical compositions of the invention may also comprise one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention, which are currently being used, have been

used, or are known to be useful in the prevention, treatment or amelioration of one or more symptoms associated with a disease or disorder.

[00293] Diseases and disorders which can be prevented, treated, managed, and/or ameliorated by administering an effective amount of one or more antibodies of the invention include, but are not limited to, diseases or disorders associated with aberrant expression and/or activity of an IL-9 polypeptide and diseases, disorders associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, autoimmune disorders (*e.g.*, lupus, rheumatoid arthritis, and multiple sclerosis), inflammatory disorders (*e.g.*, asthma, allergic disorders, and arthritis), proliferative disorders (*e.g.*, leukemia, fibrosis, carcinoma, and lymphoma), and infections (preferably, respiratory infections). In another embodiment, the antibodies of the invention and compositions comprising said antibodies are administered to a subject, preferably a human subject, according to the methods of the invention to prevent, treat, manage, and/or ameliorate one or more symptoms of a disease or disorder associated with aberrant expression and/or activity of an IL-9 polypeptide or a disease or disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof. In a specific embodiment, the antibodies of the invention and compositions comprising the same are used to prevent, treat, manage, and/or ameliorate a proliferative disorder. In another embodiment, the antibodies of the invention and compositions comprising the same are used to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In another embodiment, the antibodies of the invention and compositions comprising the same are used to prevent, treat, manage, and/or ameliorate asthma or one or more symptoms thereof. In another specific embodiment, the antibodies of the invention and compositions comprising said antibodies are used to prevent, treat, manage, and/or ameliorate an allergic condition including, but not limited to, rhinitis, eczema, chronic urticaria, atopic dermatitis, and allergic asthma. In another embodiment, the antibodies of the invention and compositions comprising the same are used to prevent, treat, manage, and/or ameliorate an autoimmune disorder. In another embodiment, the antibodies of the invention and compositions comprising the same are used to prevent, treat, manage, and/or ameliorate an infection, preferably a respiratory infection, or one or more symptoms thereof. In another embodiment, the antibodies of the invention and compositions comprising said antibodies are used to prevent, treat, manage, and/or ameliorate disorders and diseases in which overproduction or abnormal production of mucin is involved in the disease pathology. Examples of such diseases and disorders include, but not limited to, cystic fibrosis, emphysema and COPD.

### 5.3.1 Proliferative Disorders

**[00294]** The antibodies of the invention and compositions comprising said antibodies can be used to prevent, treat, manage, and/or ameliorate a proliferative disorder or one or more symptoms thereof. In a specific embodiment, the proliferative disorder is characterized by aberrant proliferation (*e.g.* uncontrolled proliferation or lack of proliferation) of cells that IL-9 mediates the growth of, including, but not limited to T cells, erythroid progenitors, B cells, mast cells, eosinophils, neutrophils, and fetal thymocytes.

**[00295]** The present invention provides methods for preventing, treating, managing, and/or ameliorating one or more symptoms of a non-cancerous disorder (*i.e.*, a disorder that does not have the potential to metastasize) associated with IL-9 mediated cellular hyperproliferation, particularly of epithelial cells (*e.g.*, as in asthma, COPD, lung fibrosis, bronchial hyperresponsiveness, psoriasis, lymphoproliferative disorder, and seborrheic dermatitis) and endothelial cells (*e.g.*, as in restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, and macular degeneration), said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. The present invention also provides methods for preventing, managing, treating, and/or ameliorating a non-cancerous disorder associated with IL-9 mediated cellular hyperproliferation, said methods comprising of administering to a subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention useful for the prevention, treatment, management, and/or amelioration of said disorder. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, and the anti-viral agents described in section 5.2.6. One or more of the antibodies of the invention may also be used in combination with an anti-cancer therapy such as radiation therapy as described in section 5.2.5.

**[00296]** The invention provides methods for preventing, treating, managing, and/or ameliorating one or more symptoms of a non-cancerous disorder associated with IL-9 mediated cellular hyperproliferation in a subject refractory to conventional therapies for such disorder, said methods comprising administering to subject an effective amount of one or more antibodies, compositions, or combination therapies of the invention. In certain embodiments, a patient with a non-cancerous disorder associated with IL-9 mediated

cellular hyperproliferation is refractory to a therapy when the hyperproliferation has not been eradicated and/or the symptoms have not been alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of non-cancerous hyperproliferation disorders, using art-accepted meanings of “refractory” such a context. In various embodiments, a patient with a non-cancerous disorder associated with IL-9 mediated cellular hyperproliferation is refractory when the patient’s levels of IL-9 remain abnormal and/or if cellular proliferation has not been decreased. The present invention also provides methods for preventing, managing, treating, and/or ameliorating a non-cancerous disorder associated with IL-9 mediated cellular hyperproliferation in a subject refractory to conventional therapies for such disorder, said methods comprising of administering to a subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention useful for the prevention, treatment, management, and/or amelioration of said disorder. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, and the anti-viral agents described in section 5.2.6. One or more of the antibodies of the invention may also be used in combination with an anti-cancer therapy such as radiation therapy as described in section 5.2.5.

**[00297]** The present invention provides methods for preventing, treating, managing, and/or ameliorating cancer or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. The invention also provides methods for preventing, treating, managing, and/or ameliorating cancer in which an effective amount of one or more antibodies of the invention are administered in combination with one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention useful for the prevention, treatment, management, or amelioration of cancer or a secondary condition associated therewith (*e.g.*, a viral, bacterial, or fungal infection). Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-

viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8. One or more of the antibodies of the invention may also be used in combination with an anti-cancer therapy such as radiation therapy as described in section 5.2.5.

**[00298]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists,” International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled “The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents,” U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating Disorders by Administering an Integrin  $\alpha_v\beta_3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety) to a subject at risk of or with a proliferative disorder. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of sipilizumab (MedImmune, Inc., International Publication No. WO 02/069904, which is incorporated herewith by reference) to a subject to prevent, treat, manage, and/or ameliorate a proliferative disorder. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)) to a subject to prevent, treat, manage, and/or ameliorate a proliferative disorder. In yet another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™, sipilizumab, and/or EphA2 inhibitor to a subject to prevent, treat, manage, and/or ameliorate a proliferative disorder. In another preferred embodiment, an antibody

derivative such as MT103, part of a class of antibody derivatives known as Bi-Specific T Cell Engagers (BiTE™; MedImmune, Inc.), may also be used in combination with one or more antibodies of the present invention.

**[00299]** The antibodies of the invention or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat, and/or ameliorate a proliferative disorder or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating a proliferative disorder or one or more symptoms thereof in a patient undergoing therapies for other disease or disorders. The invention encompasses methods of preventing, managing, treating, and/or ameliorating a proliferative disorder or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other than antibodies of the invention develops. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating a proliferative disorder or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies.

**[00300]** The invention encompasses methods for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients with cancer that are immunosuppressed as a result of having previously undergone other cancer therapies. The invention also encompasses methods for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients who have proven refractory to other therapies but are no longer on these therapies. The invention also encompasses alternative therapies for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof patients in which chemotherapy, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy has proven or may prove too toxic, *i.e.*, results in unacceptable or unbearable side effects, for the patient being undergoing said therapy. The invention also encompasses methods for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients predisposed to cancer. The invention also encompasses methods for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients with mean absolute lymphocyte cell counts of at least 500 cells/ mm<sup>3</sup>, preferably at least 600 cells/ mm<sup>3</sup>, more preferably at least 750 cells/ mm<sup>3</sup>. The invention also encompasses methods for preventing the onset or development of one or more symptoms in patients with cancer. The invention also encompasses methods to prevent, treat, manage, and/or ameliorate one or more symptoms in patients with incurable cancer, in particular hospice patients. Further, the invention provides methods for preventing cancer in patients who have been treated for cancer but have no disease activity.

**[00301]** In a preferred embodiment, the invention encompasses methods for managing for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in patients that have undergone or are undergoing chemotherapy. In accordance with this embodiment, such patients include patients that have undergone or are undergoing radiation therapy, hormonal therapy, biological therapy/immunotherapy and/or surgery. Examples of chemotherapeutic agents that are used to treat cancer include, but not limited to methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclosporin A, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbazine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, docetaxel, etc.

**[00302]** In a specific embodiment, the invention encompasses methods for preventing, managing, treating, and/or ameliorating cancer or one or more symptoms thereof in cancer patients that have undergone or are undergoing radiation therapy. In accordance with this embodiment, such patients include patients that have undergone or are undergoing chemotherapy, hormonal therapy, biological therapy/immunotherapy and/or surgery. In another embodiment, the invention encompasses methods for treating or managing patients that have undergone or are undergoing hormonal therapy and/or biological therapy/immunotherapy. In accordance with this embodiment, such patients include patients that have undergone or are undergoing chemotherapy, radiation therapy and/or surgery.

**[00303]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating a proliferative disorder or a symptom thereof in a patient who has proven refractory to therapies other than antibodies, compositions, or combination therapies of the invention. In certain embodiments, a patient with a proliferative disorder is refractory to a therapy when proliferation disorders has not been eradicated and/or the symptoms have not been alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of proliferative disorders, using art-accepted meanings of “refractory” such a context. In various embodiments, a patient with a proliferative disorder is refractory when the patient’s levels of IL-9 remain abnormal and/or if cellular proliferation has not been decreased.

**[00304]** The present invention provides methods for preventing, treating, managing, and/or ameliorating a proliferative disorder or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being

managed or treated in accordance with the methods of the invention is to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease), a person with impaired renal or liver function, the elderly, children, infants, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact with conventional agents used to manage or treat a proliferative disorder.

**[00305]** The invention encompasses methods for preventing, managing, treating, and/or ameliorating one or more symptoms of a cancer in a subject refractory to existing single agent therapies for such a cancer. The invention also encompasses methods for preventing, treating, managing, and/or ameliorating cancer or a secondary condition associated thereof in patients who have proven refractory to other therapies but are no longer on these therapies. The invention also encompasses methods for the prevention, treatment, management, or amelioration of cancer in a patient immunosuppressed by reason of having previously undergone other cancer therapies. The invention provides alternative methods for the prevention, treatment, management, or amelioration of cancer where chemotherapy, radiation therapy, hormonal therapy, and/or biological therapy/immunotherapy has proven or may prove too toxic, *i.e.*, results in unacceptable or unbearable side effects, for the subject being undergoing therapy. Further, the invention encompasses methods for preventing the recurrence of cancer in patients that have been treated and have no disease.

**[00306]** Cancers that can be treated by the methods encompassed by the invention include, but are not limited to, neoplasms, tumors, metastases, or any disease or disorder characterized by uncontrolled cell growth. The cancer may be a primary or metastatic cancer. Specific examples of cancers that can be treated by the methods encompassed by the invention include, but are not limited to, cancer of the head, neck, eye, mouth, throat, esophagus, chest, bone, lung, colon, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, and brain. Additional cancers include, but are not limited to, the following: leukemias such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia,



solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone cancer and connective tissue sarcomas such as but not limited to bone sarcoma, myeloma bone disease, multiple myeloma, cholesteatoma-induced bone osteosarcoma, Paget's disease of bone, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, and synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, and primary brain lymphoma; breast cancer including but not limited to adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease (including juvenile Paget's disease) and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytoma and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipidus; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma, gallbladder cancers such as

adenocarcinoma; cholangiocarcinomas such as but not limited to pappillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and verrucous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America). It is also contemplated that cancers caused by aberrations in apoptosis can also be treated by the methods and compositions of the invention. Such cancers may include, but not be limited to, follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes.

**[00307]** Therapies and dosages, routes of administration, and recommended usage of therapies for preventing, treating, managing, and/or ameliorating proliferative disorders or one or more symptoms thereof are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

### **5.3.2 Inflammatory Disorders**

**[00308]** One or more antibodies of the invention and compositions comprising of said antibodies can be used to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. The antibodies of the invention or compositions comprising said antibodies may also be administered in combination with one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents other than antibodies of the invention) useful for the prevention, treatment, management, and/or amelioration of an inflammatory disorder or one or more symptoms thereof. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

**[00309]** In a specific embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof of an effective amount of one or more of the antibodies of the invention and an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention.

**[00310]** The invention provides methods for preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof in a subject refractory to conventional therapies (*e.g.*, methotrexate and a TNF- $\alpha$  antagonist (*e.g.*, REMICADE™ or ENBREL™)) for such an inflammatory disorder, said methods comprising administering to said subject an effective amount of one or more antibodies of the invention. The invention also provides methods for preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof in a subject refractory to existing single agent therapies for such an inflammatory disorder, said methods comprising administering to said subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention. The invention also provides methods for preventing, treating, managing, and/or ameliorating an

inflammatory disorder in patients who have proven refractory to other therapies but are no longer on these therapies. The invention also provides alternative methods for the prevention, treatment, management, and/or amelioration of an inflammatory disorder where another therapy has proven or may prove too toxic, *i.e.*, results in unacceptable or unbearable side effects, for the subject being treated. Further, the invention provides methods for preventing the recurrence of an inflammatory disorder in patients that have been treated and have no disease activity.

**[00311]** Examples of the inflammatory disorders which can be prevented, managed, treated, and/or ameliorated in accordance with the methods of the invention, include, but are not limited to, asthma, allergic disorders, inflammatory disorders characterized by type-2 mediated inflammation, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), encephalitis, inflammatory bowel disease, septic shock, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacteria infections. In a preferred embodiment, the inflammatory disorder which is prevented, treated, managed, and/or ameliorated in accordance with the methods of the invention is an inflammatory disorder characterized as a type 2-mediated inflammation. Type 2-mediated inflammation is characterized by eosinophilic and basophilic tissue infiltration and/or extensive mast cell degranulation, a process dependent on cross-linking of surface-bound IgE. In another preferred embodiment, the inflammatory disorder which is prevented, treated, managed, and/or ameliorated in accordance with the methods of the invention is asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, or an allergic disorder.

**[00312]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject to prevent, treat, manage, and/or ameliorate asthma or one or more symptoms thereof in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention useful, in preventing, treating, managing, and/or ameliorating asthma or one or more symptoms thereof. Non-limiting examples of such therapies include agents such as adrenergic stimulants (*e.g.*, catecholamines (*e.g.*, epinephrine, isoproterenol, and isoetharine), resorcinols (*e.g.*, metaproterenol, terbutaline, and fenoterol), saligenins (*e.g.* salbutamol)), anticholinergics (*e.g.*, atropine sulfate, atropine methylnitrate, and ipratropium bromide (ATROVENT™)), beta2-agonists (*e.g.* albuterol (VENTOLIN™ and PROVENTIL™), bitolterol (TORNALATE™), levalbuterol (XOPONEX™), metaproterenol (ALUPENT™), pirbuterol (MAXAIR™), terbutaline (BRETHAIRE™ and BRETHINE™), albuterol (PROVENTIL™, REPETABS™, and VOLMAX™), formoterol

(FORADIL AEROLIZER™), and salmeterol (SEREVENT™ and SEREVENT DISKUS™)), corticosteroids (*e.g.*, methyprednisolone (MEDROL™), prednisone (PREDNISONONE™ and DELTASONE™), and prednisolone (PRELONE™, PEDIAPRED™)), glucocorticoids (*e.g.* oral steroids or other systemic or oral steroids, and inhaled glucocorticoids), other steroids, immunosuppressant agents (*e.g.* methotrexate and gold salts), leukotriene inhibitors (*e.g.*, montelukast (SINGULAIR™), zafirlukast (ACCOLATE™), and zileuton (ZYFLO™)), mast cell modulators (*e.g.*, cromolyn sodium (INTAL™) and nedocromil sodium (TILADE™)), methylxanthines (*e.g.*, theophylline (UNIPHYL™, THEO-DUR™, SLO-BID™, AND TEHO-42™)), and mucolytic agents (*e.g.*, acetylcysteine)). In a specific embodiment, one or more antibodies of the invention are administered in combination with siplizumab (MedImmune, Inc.) to a subject to prevent, treat, manage, and/or ameliorate asthma or one or more symptoms thereof.

**[00313]** The invention encompasses methods of preventing the development of asthma in a patient expected to suffer from, or at risk of developing asthma, *e.g.*, patients with genetic disposition for asthma, patients who have or have had one or more respiratory infections, infants, infants born prematurely, children, the elderly, or patients who work with toxic chemicals (*i.e.*, at risk of developing occupational asthma). In specific embodiments, the subjects are children who are at risk of developing asthma, *e.g.*, children who have or have had a respiratory infection, particularly, PIV, RSV, and hMPV, have elevated IgE levels, a family history of asthma, have been exposed to asthma triggers and/or allergens (*e.g.*, animals, cockroach allergens, and tobacco smoke), or have experienced wheezing or bronchial hyperresponsiveness. For a discussion of risk factors for asthma, see, *e.g.*, Klinnert *et al.*, 2001, *Pediatrics* 108(4):E69; London *et al.*, 2001, *Epidemiology*, 12(5):577-83; Melen *et al.*, 2001, *Allergy*, 56(7): 464-52; Mochizuki *et al.*, 2001, *J Asthma* 38(1):1-21; Arruda *et al.*, 2001, *Curr Opin Pulm Med*, 7(1):14-19; Castro-Rodriguez *et al.*, 2000, *Am J Respir Crit Care Med* 162: 1403-6; Gold, 2000, *Environ Health Perspect* 108: 643-51; and Csonka *et al.*, 2000, *Pediatr Allergy Immuno*, 11(4): 225-9.

**[00314]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms thereof in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) useful in preventing, treating, managing, and/or ameliorating allergies or one or more symptoms thereof. Non-limiting examples of such therapies include agents such as antimediator drugs (*e.g.*, antihistamine, *see* Table 5), corticosteroids, decongestants, sympathomimetic drugs (*e.g.*,  $\alpha$ -

adrenergic and  $\beta$ -adrenergic drugs), theophylline and its derivatives, glucocorticoids, and immunotherapies (*e.g.*, repeated long-term injection of allergen, short course desensitization, and venom immunotherapy).

**Table 5. H<sub>1</sub> Antihistamines**

Chemical class and representative drugs	Usual daily dosage
Ethanolamine Diphenhydramine Clemastine	25-50 mg every 4-6 hours 0.34-2.68 mg every 12 hours
Ethylenediamine Tripeleminamine	25-50 mg every 4-6 hours
Alkylamine Brompheniramine Chlorpheniramine Triprolidine (1.25 mg/5ml)	4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hour 4 mg every 4-6 hours; or 8-12 mg of SR form every 8-12 hours 2.5 mg every 4-6 hours
Phenothiazine Promethazine	25 mg at bedtime
Piperazine Hydroxyzine	25 mg every 6-8 hours
Piperidines Astemizole (nonsedating) Azatadine Cetirizine Cyproheptadine Fexofenadine (nonsedating) Loratidine (nonsedating)	10 mg/d 1-2 mg every 12 hours 10 mg/d 4 mg every 6-8 hour 60 mg every 12 hours 10 mg every 24 hours

**[00315]** In one embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered in combination with an effective amount of one or more anti-IgE antibodies to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered with an effective amount of anti-IgE antibody TNX901 to a subject to prevent, treat, manage, and/or ameliorate allergy or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered with an effective amount of anti-IgE antibody rhuMAb-E25 omalizumab to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered in combination with an effective amount of anti-IgE antibody

HMK-12 to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms. In another embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered in combination with an effective amount of anti-IgE antibody 6HD5 to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms. In another embodiment, an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide is administered in combination with an effective amount of anti-IgE antibody MAb Hu-901 to a subject at risk of or with allergies to a subject to prevent, treat, manage, and/or ameliorate an allergy or one or more symptoms.

**[00316]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject in combination with an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents) other than an antibody that immunospecifically binds to an IL-9 polypeptide that useful in preventing, treating, managing, and/or ameliorating COPD or one or more symptoms thereof. Non-limiting examples of such therapies include agents such as bronchodilators (*e.g.* short-acting  $\beta_2$ -adrenergic agonist (*e.g.*, albuterol, pirbuterol, terbutaline, and metaproterenol), long-acting  $\beta_2$ -adrenergic agonists (*e.g.*, oral sustained-release albuterol and inhaled salmeterol), anticholinergics (*e.g.*, ipratropium bromide), and theophylline and its derivatives (therapeutic range for theophylline is preferably 10 - 20  $\mu\text{g/mL}$ ), glucocorticoids, exogenous  $\alpha_1\text{AT}$  (*e.g.*,  $\alpha_1\text{AT}$  derived from pooled human plasma administered intravenously in a weekly dose of 60 mg/kg ), oxygen, lung transplantation, lung volume reduction surgery, endotracheal intubation, ventilation support, yearly influenza vaccine and pneumococcal vaccination with 23-valent polysaccharide, exercise, and smoking cessation.

**[00317]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject in need thereof in combination with an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents) other than antibodies of the invention useful in preventing, treating, managing, and/or ameliorating pulmonary fibrosis or one or more symptoms thereof. Non-limiting examples of such therapies include, oxygen, corticosteroids (*e.g.*, daily administration of prednisone beginning at 1-1.5 mg/kg/d (up to 100 mg/d) for six weeks and tapering slowly over 3 - 6 months to a minimum maintenance dose of 0.25 mg/kg/d), cytotoxic drugs (*e.g.*, cyclophosphamide at 100 - 120 mg orally once daily and azathioprine at 3 mg/kg up to 200 mg orally once daily), bronchodilators (*e.g.*, short- and long- acting  $\beta_2$ -adrenergic agonists, anticholinergics, and theophylline and its derivatives), and antihistamines (*e.g.*, diphenhydramine and doxylamine).

**[00318]** In a specific embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists,” International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled “The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents,” U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating Disorders by Administering an Integrin  $\alpha_v\beta_3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety) to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of sipilizumab (MedImmune, Inc., International Publication No. WO 02/069904) to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)) to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In yet another preferred embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™, sipilizumab, and/or EphA2 inhibitor to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof.

**[00319]** In one embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of a stem cell factor (c-



kit ligand) inhibitor, such as, but not limited to MAb 7H6, MAb 8H7a, pAb 1337, FK506, and CsA to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In accordance with this embodiment, the stem cell factor inhibitor is preferably administered locally in the affected area (*i.e.*, the inflamed area). In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more c-kit receptor inhibitors, such as, but not limited to STI 571 to a subject to prevent, treat, manage, and/or ameliorate an inflammatory disorder or one or more symptoms thereof. In accordance with this embodiment, the c-kit receptor inhibitor is preferably administered locally in the affected area.

**[00320]** In one embodiment, an effective amount of one or more antibodies of the invention is administered in combination with a mast cell protease inhibitor to a subject at risk of or with an inflammatory disorder. In another embodiment, the mast cell protease inhibitor is a tryptase kinase inhibitor, such as, but not limited to GW-45, GW-58, and genisteine. In a specific embodiment, the mast cell protease inhibitor is phosphatidylinositol-3' (PI3)-kinase inhibitors, such as, but not limited to calphostin C. In another embodiment, the mast cell protease inhibitor is a protein kinase inhibitor such as, but not limited to staurosporine. In accordance with this embodiment, the mast cell protease inhibitor is preferably administered locally to the affected area.

**[00321]** The antibodies of the invention or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat, and/or ameliorate an inflammatory disorder or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof in a patient undergoing therapies for other disease or disorders. The invention encompasses methods of preventing, managing, treating, and/or ameliorating an inflammatory disorder or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other than antibodies of the invention develops. The invention also encompasses methods of preventing, treating, managing, and/or ameliorating an inflammatory disorder or a symptom thereof in refractory patients. The invention encompasses methods for preventing, treating, managing, and/or ameliorating a proliferative disorder or a symptom thereof in a patient who has proven refractory to therapies other than antibodies, compositions, or combination therapies of the invention. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of proliferative disorders, using art-accepted meanings of "refractory" such a

context. In certain embodiments, a patient with an inflammatory disorder is refractory to a therapy when inflammation is not prevented, managed, and/or alleviated. The invention further encompasses methods of preventing, managing, treating, and/or ameliorating an inflammatory disorder or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies.

**[00322]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating an inflammatory disorder or a symptom thereof in a patient who has proven refractory to therapies other than antibodies that immunospecifically bind to an IL-9 polypeptide but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with anti-inflammatory agents, antibiotics, anti-viral therapy, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring inflammatory disorders despite management or treatment with existing therapies.

**[00323]** The present invention encompasses methods for preventing, treating, managing, and/or ameliorating an inflammatory disorder or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being managed or treated in accordance with the methods of the invention is refractory to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease, patients with broncho-pulmonary dysplasia, patients with congenital heart disease, patients with cystic fibrosis, patients with acquired or congenital heart disease, and patients suffering from an infection), a person with impaired renal or liver function, the elderly, children, infants, infants born prematurely, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact with conventional agents used to prevent, manage, treat, or ameliorate a viral respiratory infection or one or more symptoms thereof.

**[00324]** Anti-inflammatory therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

### **5.3.3 Autoimmune Disorders**

**[00325]** One or more antibodies of the invention and compositions comprising said antibodies can be used to prevent, treat, manage, and/or ameliorate an autoimmune disorder

or one or more symptoms thereof. An effective amount of one or more of the antibodies of the invention may also be administered to a subject to prevent, manage, treat, and/or ameliorate an autoimmune disorder or one or more symptoms thereof in combination with an effective amount one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention which are useful for the prevention, treatment, management, and/or amelioration of an autoimmune disorder, to a subject in need thereof to prevent, treat, manage, and/or ameliorate an autoimmune disorder or one or more symptoms thereof. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

**[00326]** In a specific embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating an autoimmune disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof a dose of an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating an autoimmune disorder or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention.

**[00327]** In autoimmune disorders, the immune system triggers an immune response when there are no foreign substances to fight and the body's normally protective immune system causes damage to its own tissues by mistakenly attacking self. There are many different autoimmune disorders which affect the body in different ways. For example, the brain is affected in individuals with multiple sclerosis, the gut is affected in individuals with Crohn's disease, and the synovium, bone and cartilage of various joints are affected in individuals with rheumatoid arthritis. As autoimmune disorders progress destruction of one or more types of body tissues, abnormal growth of an organ, or changes in organ function may result. The autoimmune disorder may affect only one organ or tissue type or may affect multiple organs and tissues. Organs and tissues commonly affected by autoimmune disorders include red blood cells, blood vessels, connective tissues, endocrine glands (*e.g.*, the thyroid or pancreas), muscles, joints, and skin. Examples of autoimmune disorders that

can be prevented, treated, managed, and/or ameliorated by the methods of the invention include, but are not limited to, adrenergic drug resistance, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, allergic encephalomyelitis, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inflammatory eye disease, autoimmune neonatal thrombocytopenia, autoimmune neutropenia, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, autoimmune thyroiditis, Behcet's disease, bullous pemphigoid, cardiomyopathy, cardiomyopathy syndrome, celiac sprue-dermatitis, chronic active hepatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, dense deposit disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis (*e.g.*, IgA nephropathy), gluten-sensitive enteropathy, Goodpasture's syndrome, Graves' disease, Guillain-Barre, hyperthyroidism (*i.e.*, Hashimoto's thyroiditis), idiopathic pulmonary fibrosis, idiopathic Addison's disease, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erythematosus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, Myasthenia Gravis, myocarditis, type 1 or immune-mediated diabetes mellitus, myasthenia gravis, myocarditis, neuritis, other endocrine gland failure, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, Polyendocrinopathies, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, post-MI, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, relapsing polychondritis, Reiter's syndrome, rheumatic heart disease, rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, urticaria, uveitis, Uveitis Ophthalmia, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

**[00328]** In specific embodiment, an effective amount of one or more antibodies of the invention is administered to prevent, treat, manage, and/or ameliorate multiple sclerosis or one or more symptoms thereof in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, useful in preventing, treating, managing, and/or ameliorating multiple sclerosis (MS) or one or more symptoms thereof. Non-limiting examples of such therapies include agents such as IFN- $\beta$ 1b (BETSERON®) (*e.g.*, 8.0 million international unites (MIU) is administered by subcutaneous injection every other day), IFN- $\beta$ 1a (AVONEX®) (*e.g.*, 6.0

MIU is administered by intramuscular injection once every week), glatiramer acetate (COPAXONE®) (*e.g.*, 20 mg is administered by subcutaneous injection every day), mitoxantrone (*e.g.*, 12 mg/m<sup>2</sup> is administered by intravenous infusion every third month), azathioprine (*e.g.*, 2-3 mg/kg body weight is administered orally each day), methotrexate (*e.g.*, 7.5 mg is administered orally once each week), cyclophosphamide, intravenous immunoglobulin (*e.g.*, 0.15 - 0.2 g/kg body weight administered monthly for up to 2 years), glucocorticoids, methylprednisolone (*e.g.*, administered in bimonthly cycles at high doses), 2-chlorodeoxyadenosine (*e.g.*, cladribine (LEUSTATIN®), baclofen (*e.g.*, 15 to 80 mg/d in divided doses, or orally in higher doses up to 240 mg/d, or intrathecally via an indwelling catheter), cycloenzaprine hydrochloride (*e.g.*, 5-10 mg bid or tid), clonazepam (*e.g.*, 0.5 to 1.0 mg tid, including bedtime dose), clonidine hydrochloride (*e.g.*, 0.1 to 0.2 mg tid, including a bedtime dose), carbamazepine (*e.g.*, 100-1200 mg/d in divided, escalating doses), gabapentin (*e.g.*, 300-3600 mg/d), dilantin (*e.g.*, 300 - 400 mg/d), amitriptyline (*e.g.*, 25 - 150 mg/d), baclofen (*e.g.*, 10 - 80 mg/d), primidone (*e.g.*, 125 - 250 mg bid or tid), ondansetron (*e.g.*, 4 to 8 mg bid or tid), isoniazid (*e.g.*, up to 1200 mg in divided doses), oxybutynin (*e.g.*, 5 mg bid or tid), tolterodine (*e.g.*, 1 - 2 mg bid), propantheline (*e.g.*, 7.5 to 15 mg qid), bethanecol (*e.g.*, 10 - 50 mg tid or qid), terazosin hydrochloride (*e.g.*, 1-5 mg at bedtime), sildenafil citrate (*e.g.*, 50 - 100 mg po prn), amantadine (*e.g.*, 100 mg bid), pemoline (*e.g.*, 37.5 mg bid), high dose vitamins, calcium orotate, gancyclovir, antibiotic, and plasma exchange.

**[00329]** In specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject to prevent, manage, treat, and/or ameliorate psoriasis or one or more symptoms thereof in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, useful in preventing, treating, managing, and/or ameliorating psoriasis or one or more symptoms thereof. Non-limiting examples of such therapies include agents such as a topical steroid cream or ointment, tar (*e.g.*, ESTAR®, PSORIGEL®, FOTOTAR®, and LCD 10% in Nutraderm lotion or mixed directly with triamcinolone 0.1% cream)), occlusion, topical vitamin D analogue (*e.g.*, calcipotriene ointment), ultraviolet light PUVA (psoralen plus ultraviolet A), methotrexate (*e.g.*, up to 25 mg once weekly or in divided doses every 12 hours for three doses once a week), synthetic retinoid (*e.g.*, etretinate in dosage of 0.5-1 mg/kg/d), immunomodulatory therapy (*e.g.*, cyclosporine); and sulfasalazine (*e.g.*, in dosages of 1 g three times daily).

**[00330]** In specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject to prevent, treat, manage, and/or ameliorate Crohn's

disease or a symptom thereof in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, useful in preventing, treating, managing, and/or ameliorating Crohn's disease or one or more symptoms thereof. Non-limiting examples of such therapies include antidiarrheals (*e.g.*, loperamide 2-4 mg up to 4 times a day, diphenoxylate with atropine 1 tablet up to 4 times a day, tincture of opium 8-15 drops up to 4 times a day, cholestyramine 2-4 g or colestipol 5 g once or twice daily), antispasmodics (*e.g.*, propantheline 15 mg, dicyclomine 10 -20 mg, or hyoscyamine 0.125 mg given before meals), 5-aminosalicylic acid agents (*e.g.*, sulfasalazine 1.5-2 g twice daily, mesalamine (ASACOL®) and its slow release form (PENTASA®), especially at high dosages, *e.g.*, PENTASA® 1g four times daily and ASACOL® 0.8 - 1.2 g four times daily), corticosteroids, immunomodulatory drugs (*e.g.*, azathioprine (1 -2 mg/kg), mercaptopurine (50 - 100 mg), cyclosporine, and methotrexate), antibiotics, TNF inhibitors (*e.g.*, inflixmab (REMICADE®)), immunosuppressive agents (*e.g.*, tacrolimus, mycophenolate mofetil, and thalidomide), anti-inflammatory cytokines (*e.g.*, IL-10 and IL-11), nutritional therapies, enteral therapy with elemental diets (*e.g.*, Vivonex for 4 weeks), and total parenteral nutrition.

**[00331]** In another specific embodiment, an effective amount of one or more antibodies of the invention is administered to a subject to prevent, treat, manage, and/or ameliorate lupus erythematosus or one or more symptoms thereof in combination with an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention, useful in preventing, treating, managing, and/or ameliorating lupus erythematosus or one or more symptoms thereof. Non-limiting examples of such therapies include antimalarials (*e.g.*, hydroxychloroquine), glucocorticoids (*e.g.*, low dose, high dose, or high-dose intravenous pulse therapy), immunosuppressive agents (*e.g.*, cyclophosphamide, chlorambucil, and azathioprine), cytotoxic agents (*e.g.*, methotrexate and mycophenolate mofetil), androgenic steroids (*e.g.*, danazol), and anticoagulants (*e.g.*, warfarin).

**[00332]** In a specific embodiment, an prophylactically or therapeutically effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled "Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists," International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled "The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents," U.S. Patent

Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating Disorders by Administering an Integrin  $\alpha_v\beta_3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety) to a subject to prevent, treat, manage, and/or ameliorate an autoimmune disorder or one or more symptoms thereof. In another preferred embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of siplizumab (MedImmune, Inc., International Publication No. WO 02/069904) to a subject to prevent, treat, manage, and/or ameliorate an autoimmune disorder or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)) to a subject to prevent, treat, manage, and/or ameliorate an autoimmune disorder or one or more symptoms thereof. In yet another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of VITAXIN™, siplizumab, and/or EphA2 inhibitor to a subject to prevent, treat, manage, and/or ameliorate an autoimmune disorder or one or more symptoms thereof.

**[00333]** The antibodies of the invention or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat, and/or ameliorate an autoimmune disorder or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating an autoimmune disorder or one or more symptoms thereof in a patient undergoing therapies for other disease or disorders. The invention encompasses methods of preventing, managing, treating, and/or ameliorating an autoimmune disorder or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other than antibodies of the invention develops. The invention also encompasses methods of preventing, treating, managing, and/or ameliorating an autoimmune disorder or a symptom thereof in refractory

patients. The invention encompasses methods for preventing, treating, managing, and/or ameliorating a proliferative disorder or a symptom thereof in a patient who has proven refractory to therapies other than antibodies, compositions, or combination therapies of the invention. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of autoimmune disorders, using art-accepted meanings of “refractory” such a context. In certain embodiments, a patient with an autoimmune disorder is refractory to a therapy when one or more symptoms of an autoimmune disorder is not prevented, managed, and/or alleviated. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating an autoimmune disorder or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies.

**[00334]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating an proliferative disorder or a symptom thereof in a patient who has proven refractory to therapies other than antibodies that immunospecifically bind to an IL-9 polypeptide but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with anti-inflammatory agents, immunomodulatory agents, antibiotics, anti-viral therapy, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring allergic reactions despite management or treatment with existing therapies.

**[00335]** The present invention encompasses methods for preventing, treating, managing, and/or ameliorating an autoimmune disorder or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being managed or treated in accordance with the methods of the invention is refractory to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease, patients with broncho-pulmonary dysplasia, patients with congenital heart disease, patients with cystic fibrosis, patients with acquired or congenital heart disease, and patients suffering from an infection), a person with impaired renal or liver function, the elderly, children, infants, infants born prematurely, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact with conventional agents used to prevent, manage, treat, or ameliorate a viral respiratory infection or one or more symptoms thereof.



**[00336]** Autoimmune therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

#### **5.3.4 Viral Infections**

**[00337]** One or more antibodies of the invention and compositions comprising said antibodies can be administered to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. One or more antibodies of the invention and compositions comprising said antibodies may be administered in combination with one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention useful for the prevention, treatment, management, or amelioration of a viral infection to a subject predisposed to or with a viral infection, preferably a respiratory viral infection. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-angiogenic agents described in section 5.2.2, the TNF- $\alpha$  antagonists described in section 5.2.3, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

**[00338]** In a specific embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a viral respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a viral respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention.

**[00339]** In certain embodiments, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) currently being used, have been used, or are known to be useful in the prevention, management, treatment, and/or amelioration of a viral infection, preferably a viral respiratory infection, or one or more symptoms thereof to a subject in need thereof. Therapies for a viral infection, preferably a viral respiratory infection include, but are not limited to, anti-viral agents such as amantadine, oseltamivir,

ribavirin, palivizumab, and anamivir. In certain embodiments, an effective amount of one or more antibodies of the invention is administered in combination with one or more supportive measures to a subject in need thereof to prevent, manage, treat, and/or ameliorate a viral infection or one or more symptoms thereof. Non-limiting examples of supportive measures include humidification of the air by an ultrasonic nebulizer, aerosolized racemic epinephrine, oral dexamethasone, intravenous fluids, intubation, fever reducers (*e.g.*, ibuprofen, acetaminophen), and antibiotic and/or anti-fungal therapy (*i.e.*, to prevent or treat secondary bacterial infections).

**[00340]** Any type of viral infection or condition resulting from or associated with a viral infection (*e.g.*, a respiratory condition) can be prevented, treated, managed, and/or ameliorated in accordance with the methods of the invention, said methods comprising administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of another therapy (*e.g.*, a prophylactic or therapeutic agent other than antibodies of the invention). Examples of viruses which cause viral infections include, but are not limited to, retroviruses (*e.g.*, human T-cell lymphotropic virus (HTLV) types I and II and human immunodeficiency virus (HIV)), herpes viruses (*e.g.*, herpes simplex virus (HSV) types I and II, Epstein-Barr virus, HHV6-HHV8, and cytomegalovirus), arenaviruses (*e.g.*, lassa fever virus), paramyxoviruses (*e.g.*, morbillivirus virus, human respiratory syncytial virus, mumps, hMPV, and pneumovirus), adenoviruses, bunyaviruses (*e.g.*, hantavirus), coronaviruses, filoviruses (*e.g.*, Ebola virus), flaviviruses (*e.g.*, hepatitis C virus (HCV), yellow fever virus, and Japanese encephalitis virus), hepadnaviruses (*e.g.*, hepatitis B viruses (HBV)), orthomyxoviruses (*e.g.*, influenza viruses A, B and C and PIV), papovaviruses (*e.g.*, papillomaviruses), picornaviruses (*e.g.*, rhinoviruses, enteroviruses and hepatitis A viruses), poxviruses, reoviruses (*e.g.*, rotaviruses), togaviruses (*e.g.*, rubella virus), and rhabdoviruses (*e.g.*, rabies virus). Biological responses to a viral infection include, but not limited to, elevated levels of IgE antibodies, increased proliferation and/or infiltration of T cells, increased proliferation and/or infiltration of B cells, epithelial hyperplasia, and mucin production. In a specific embodiment, the invention also provides methods of preventing, treating, managing, and/or ameliorating viral respiratory infections that are associated with or cause the common cold, viral pharyngitis, viral laryngitis, viral croup, viral bronchitis, influenza, parainfluenza viral diseases ("PIV") diseases (*e.g.*, croup, bronchiolitis, bronchitis, pneumonia), respiratory syncytial virus ("RSV") diseases, metapneumavirus diseases, and adenovirus diseases (*e.g.*, febrile respiratory disease, croup, bronchitis, pneumonia), said method comprising

administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of another therapy.

**[00341]** In a specific embodiment, influenza virus infections, PIV infections, hMPV infections, adenovirus infections, and/or RSV infections, or one or more of symptoms thereof are prevented, treated, managed, and/or ameliorated in accordance with the methods of the invention. In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a RSV respiratory infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention alone or in combination with one or more anti-viral agents such as, but not limited to, amantadine, rimantadine, oseltamivir, zanamivir, ribavirin, RSV-IVIG (*i.e.*, intravenous immune globulin infusion) (RESPIGAM™), and palivizumab. In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a PIV infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of one or more anti-viral agents such as, but not limited to, amantadine, rimantadine, oseltamivir, zanamivir, ribavirin, and palivizumab. In another specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a hMPV infection or one or more symptoms thereof, said methods comprising of administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of one or more anti-viral agents, such as, but not limited to, amantadine, rimantadine, oseltamivir, zanamivir, ribavirin, and palivizumab to a subject in need thereof. In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating influenza, said methods comprising administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of an anti-viral agent such as, but not limited to zanamivir (RELENZA®), oseltamivir (TAMIFLU®), rimantadine, and amantadine (SYMADINE®; SYMMETREL®) to a subject in need thereof.

**[00342]** The invention provides methods for preventing the development of asthma in a subject who suffers from or had suffered from a viral respiratory infection, said methods comprising administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of another therapy. In a specific embodiment, the subject is an infant born prematurely, an infant, or a child. In another specific embodiment, the subject suffered from or suffers from RSV infection.

**[00343]** In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating one or more secondary responses to a primary viral infection, said methods comprising of administering an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of other therapies (*e.g.*, other prophylactic or therapeutic agents). Examples of secondary responses to a primary viral infection, particularly a primary viral respiratory infection, include, but are not limited to, asthma-like responsiveness to mucosal stimuli, elevated total respiratory resistance, increased susceptibility to secondary viral, bacterial, and fungal infections, and development of such conditions such as, but not limited to, pneumonia, croup, and febrile bronchitis.

**[00344]** In a specific embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a viral infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists,” International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled “The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents,” U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating Disorders by Administering an Integrin  $\alpha_v\beta_3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety). In another specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a viral infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of sipilizumab (MedImmune, Inc., International Pub. No. WO 02/069904). In another embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a viral infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an

effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)). In yet another embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a viral infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™, siplizumab, and/or EphA2.

**[00345]** In one embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of one or more anti-IgE antibodies to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. In a specific embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of anti-IgE antibody TNX901 to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. In a specific embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of anti-IgE antibody rhuMAb-E25 omalizumab to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of anti-IgE antibody HMK-12 to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. In a specific embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of anti-IgE antibody 6HD5 to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered in combination with an effective amount of anti-IgE antibody MAb Hu-901 to a subject to prevent, treat, manage, and/or ameliorate a viral infection or one or more symptoms thereof.

**[00346]** The invention encompasses methods for preventing the development of viral infections, preferably viral respiratory infections, in a patient expected to suffer from a viral infection or at increased risk of such an infection, *e.g.*, patients with suppressed immune systems (*e.g.*, organ-transplant recipients, AIDS patients, patients undergoing

chemotherapy, the elderly, infants born prematurely, infants, children, patients with carcinoma of the esophagus with obstruction, patients with tracheobronchial fistula, patients with neurological diseases (*e.g.*, caused by stroke, amyotrophic lateral sclerosis, multiple sclerosis, and myopathies), and patients already suffering from a respiratory infection). The patients may or may not have been previously treated for a respiratory infection.

**[00347]** The antibodies of the invention, compositions, or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat, and/or ameliorate a viral infection, preferably a viral respiratory infection, or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating a viral infection, preferably a viral respiratory infection, or one or more symptoms thereof in a patient undergoing therapies for other diseases or disorders associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, disease or disorders associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, inflammatory disorders, autoimmune disorders, proliferative disorders, or infections (preferably, respiratory infections). The invention encompasses methods of preventing, managing, treating, and/or ameliorating a viral infection, preferably a viral respiratory infection, or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other than antibodies of the invention develops. The invention also encompasses methods of preventing, treating, managing, and/or ameliorating a viral infection, preferably a viral respiratory infection, or a symptom thereof in refractory patients. In certain embodiments, a patient with a viral infection, preferably a viral respiratory infection, is refractory to a therapy when the infection has not significantly been eradicated and/or the symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of infections, using art-accepted meanings of “refractory” in such a context. In various embodiments, a patient with a viral respiratory infection is refractory when viral replication has not decreased or has increased. The invention also encompasses methods of preventing the onset or reoccurrence of viral respiratory infections in patients at risk of developing such infections. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating a viral infection, preferably a viral respiratory infection, or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies. The invention further encompasses methods for preventing, treating, managing, and/or ameliorating a viral infection, preferably a viral respiratory infection, for which no anti-viral therapy is available.

**[00348]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating a viral infection, preferably a viral respiratory infection, or a symptom thereof in a patient who has proven refractory to therapies other than antibodies of the invention but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with antibiotics, anti-virals, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring viral infections despite management or treatment with existing therapies.

**[00349]** The present invention encompasses methods for preventing, treating, managing, and/or ameliorating a viral infection, preferably a viral respiratory infection, or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being managed or treated in accordance with the methods of the invention is refractory to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease), a person with impaired renal or liver function, the elderly, children, infants, infants born prematurely, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact with conventional agents used to prevent, manage, treat, and/or ameliorate a viral infection or one or more symptoms thereof.

**[00350]** Viral infection therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

### **5.3.5 Bacterial Infections**

**[00351]** The invention provides a method of preventing, treating, managing, and/or ameliorating a bacterial infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a bacterial infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of a one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention. Non-limiting examples of such therapies include the agents described in section

5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

**[00352]** Any type of bacterial infection or condition resulting from or associated with a bacterial infection (*e.g.*, a respiratory infection) can be prevented, treated, managed, and/or ameliorated in accordance with the methods of invention. Examples of bacteria which cause bacterial infections include, but not limited to, the *Aquaspirillum* family, *Azospirillum* family, *Azotobacteraceae* family, *Bacteroidaceae* family, *Bartonella* species, *Bdellovibrio* family, *Campylobacter* species, *Chlamydia* species (*e.g.*, *Chlamydia pneumoniae*), clostridium, *Enterobacteriaceae* family (*e.g.*, *Citrobacter* species, *Edwardsiella*, *Enterobacter aerogenes*, *Erwinia* species, *Escherichia coli*, *Hafnia* species, *Klebsiella* species, *Morganella* species, *Proteus vulgaris*, *Providencia*, *Salmonella* species, *Serratia marcescens*, and *Shigella flexneri*), *Gardinella* family, *Haemophilus influenzae*, *Halobacteriaceae* family, *Helicobacter* family, *Legionallaceae* family, *Listeria* species, *Methylococcaceae* family, mycobacteria (*e.g.*, *Mycobacterium tuberculosis*), *Neisseriaceae* family, *Oceanospirillum* family, *Pasteurellaceae* family, *Pneumococcus* species, *Pseudomonas* species, *Rhizobiaceae* family, *Spirillum* family, *Spirosomaceae* family, *Staphylococcus* (*e.g.*, methicillin resistant *Staphylococcus aureus* and *Staphylococcus pyrogenes*), *Streptococcus* (*e.g.*, *Streptococcus enteritidis*, *Streptococcus fasciae*, and *Streptococcus pneumoniae*), *Vampirovibr Helicobacter* family, and *Vampirovibrio* family.

**[00353]** In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating a bacterial respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a bacterial respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of a one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-inflammatory agents described in section 5.2.4., the anti-cancer agents described in section 5.2.5, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.



**[00354]** In certain embodiments, the invention provides methods to prevent, treat, manage, and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more of the symptoms, said methods comprising administering to a subject in need thereof one or more antibodies of the invention in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, used to prevent, treat, manage, and/or ameliorate bacterial infections. Therapies for bacterial infections, particularly, bacterial respiratory infections include, but are not limited to, anti-bacterial agents (*e.g.*, aminoglycosides (*e.g.*, gentamicin, tobramycin, amikacin, netilmicin) aztreonam, cephalosporins (*e.g.*, cefaclor, cefadroxil, cephalexin, cephazolin), clindamycin, erythromycin, penicillin (*e.g.*, penicillin V, crystalline penicillin G, procaine penicillin G), spectinomycin, and tetracycline (*e.g.*, chlortetracycline, doxycycline, oxytetracycline)) and supportive respiratory therapy, such as supplemental and mechanical ventilation. In certain embodiments, one or more antibodies of the invention are administered in combination with one or more supportive measures to a subject in need thereof to prevent, manage, treat, and/or ameliorate a bacterial infection or one or more symptoms thereof. Non-limiting examples of supportive measures include humidification of air by ultrasonic nebulizer, aerosolized racemic epinephrine, oral dexamethasone, intravenous fluids, intubation, fever reducers (*e.g.*, ibuprofen, acetaminophen), and more preferably, antibiotic or anti-viral therapy (*i.e.*, to prevent or treat secondary infections).

**[00355]** The invention provides methods for preventing, managing, treating, and/or ameliorating a biological response to a bacterial infection, preferably a bacterial respiratory infection, such as, but not limited to, elevated levels of IgE antibodies, mast cell proliferation, degranulation, and/or infiltration, increased proliferation and/or infiltration of B cells, and increased proliferation and/or infiltration of T cells, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention alone or in combination with an effective amount one or more therapies (*e.g.* a prophylactic or therapeutic agent) other than antibodies of the invention. The invention also provides methods of preventing, treating, managing, and/or ameliorating respiratory conditions caused by or associated with bacterial infections, preferably bacterial respiratory infections, such as, but not limited to, pneumococcal pneumonia, pneumonia caused by aerobic gram-negative bacilli, recurrent aspiration pneumonia, legionellosis, streptococcal disease, infections caused by *Hemophilus*, whooping cough, meningitis, or tuberculosis, said methods comprising administering to a subject in need thereof an effective amount of

one or more antibodies of the invention alone or in combination with an effective amount of another therapy.

**[00356]** In a specific embodiment, the methods of the invention are utilized to prevent, treat, manage, and/or ameliorate a bacterial respiratory infection caused by *Pneumococcus*, *Mycobacteria*, aerobic gram-negative bacilli, *Streptococcus*, or *Hemophilus* or one or more symptoms thereof, said method comprising administering to a subject in need thereof of an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention.

**[00357]** In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating one or more secondary conditions or responses to a primary bacterial infection, preferably a primary bacterial respiratory infection, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of other therapies (*e.g.*, other prophylactic or therapeutic agents). Examples of secondary conditions or responses to a primary bacterial infection, particularly a bacterial respiratory infection, include, but are not limited to, asthma-like responsiveness to mucosal stimuli, elevated total respiratory resistance, increased susceptibility to secondary viral, bacterial, and fungal infections, and development of such conditions such as, but not limited to, pneumonia, croup, and febrile bronchitis.

**[00358]** In a specific embodiment, the methods of the invention are used to prevent, manage, treat, and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists,” International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled “The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents,” U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating

Disorders by Administering an Integrin  $\alpha\text{v}\beta 3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety). In another specific embodiment, the methods of the invention are used to prevent, manage, treat, and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of siplizumab (MedImmune, Inc., International Pub. No. WO 02/069904). In another embodiment, the methods of the invention are used to prevent, manage, treat, and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)). In yet another embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof, said methods comprising administering an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™, siplizumab, and/or EphA2.

**[00359]** The invention encompasses methods for preventing the development of bacterial infections, preferably bacterial respiratory infections, in a patient expected to suffer from a bacterial respiratory infection or at increased risk of such an infection, *e.g.*, patients with suppressed immune systems (*e.g.*, organ-transplant recipients, AIDS patients, patients undergoing chemotherapy, the elderly, infants born prematurely, infants, children, patients with carcinoma of the esophagus with obstruction, patients with tracheobronchial fistula, patients with neurological diseases (*e.g.*, caused by stroke, amyotrophic lateral sclerosis, multiple sclerosis, and myopathies), and patients already suffering from an infection, particularly a respiratory infection). The patients may or may not have been previously treated for an infection.

**[00360]** The antibodies of the invention or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat,

and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof in a patient undergoing therapies for other diseases or disorders. The invention encompasses methods of preventing, managing, treating, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other than antibodies of the invention develops. The invention also encompasses methods of preventing, treating, managing, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or a symptom thereof in refractory patients. In certain embodiments, a patient with a bacterial respiratory infection is refractory to a therapy when the infection has not significantly been eradicated and/or the symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of infections, using art-accepted meanings of “refractory” in such a context. In various embodiments, a patient with a bacterial respiratory infection is refractory when bacterial replication has not decreased or has increased. The invention also encompasses methods of preventing the onset or reoccurrence of a bacterial infection, preferably a bacterial respiratory infection, in patients at risk of developing such infection. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies. The invention further encompasses methods for preventing, treating, managing, and/or ameliorating bacterial infections, preferably bacterial respiratory infections, for which no anti-bacterial therapy is available.

**[00361]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or a symptom thereof in a patient who has proven refractory to therapies other than antibodies of the invention, but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with anti-inflammatory agents, antibiotics, anti-virals, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring bacterial infections despite management or treatment with existing therapies.

[00362] The present invention encompasses methods for preventing, treating, managing, and/or ameliorating a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being managed or treated in accordance with the methods of the invention is refractory to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease), a person with impaired renal or liver function, the elderly, children, infants, infants born prematurely, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact with conventional agents used to prevent, manage, treat, and/or ameliorate a bacterial infection, preferably a bacterial respiratory infection, or one or more symptoms thereof.

[00363] Bacterial infection therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

### **5.3.6 Fungal Infections**

[00364] One or more antibodies of the invention can be administered according to methods of the invention to a subject to prevent, treat, manage, and/or ameliorate a fungal infection or one or more symptoms thereof. One or more antibodies of the invention may be also administered to a subject to treat, manage, and/or ameliorate a fungal infection and/or one or more symptoms thereof in combination with one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention which are useful for the prevention, treatment, management, or amelioration of a fungal infection or one or more symptoms thereof. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-inflammatory agents described in section 5.2.4, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

[00365] In a specific embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a fungal infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a fungal infection

or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of a one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention.

[00366] Any type of fungal infection or condition resulting from or associated with a fungal infection (*e.g.*, a respiratory infection) can be prevented, treated, managed, and/or ameliorated in accordance with the methods of invention. Examples of fungus which cause fungal infections include, but not limited to, *Absidia* species (*e.g.*, *Absidia corymbifera* and *Absidia ramosa*), *Aspergillus* species, (*e.g.*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus*), *Basidiobolus ranarum*, *Blastomyces dermatitidis*, *Candida* species (*e.g.*, *Candida albicans*, *Candida glabrata*, *Candida kerr*, *Candida krusei*, *Candida parapsilosis*, *Candida pseudotropicalis*, *Candida quillermondii*, *Candida rugosa*, *Candida stellatoidea*, and *Candida tropicalis*), *Coccidioides immitis*, *Conidiobolus* species, *Cryptococcus neoforms*, *Cunninghamella* species, dermatophytes, *Histoplasma capsulatum*, *Microsporum gypseum*, *Mucor pusillus*, *Paracoccidioides brasiliensis*, *Pseudallescheria boydii*, *Rhinosporidium seeberi*, *Pneumocystis carinii*, *Rhizopus* species (*e.g.*, *Rhizopus arrhizus*, *Rhizopus oryzae*, and *Rhizopus microsporus*), *Saccharomyces* species, *Sporothrix schenckii*, zygomycetes, and classes such as *Zygomycetes*, *Ascomycetes*, the *Basidiomycetes*, *Deuteromycetes*, and *Oomycetes*.

[00367] In a specific embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a fungal respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a fungal respiratory infection or one or more symptoms thereof, said method comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention and an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than antibodies of the invention. Non-limiting examples of such therapies include the agents described in section 5.2, *supra*, and in particular, the immunomodulatory agents described in section 5.2.1, the anti-inflammatory agents described in section 5.2.4, the anti-viral agents described in section 5.2.6, the anti-bacterial agents described in section 5.2.7, and the anti-fungal agents described in section 5.2.8.

**[00368]** In certain embodiments, an effective amount of one or more antibodies is administered in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than antibodies of the invention, which are currently being used, have been used, or are known to be useful in the prevention, management, treatment, or amelioration of a fungal infection, preferably a fungal respiratory infection, to a subject in need thereof. Therapies for fungal infections include, but are not limited to, anti-fungal agents such as azole drugs *e.g.*, miconazole, ketoconazole (NIZORAL®), caspofungin acetate (CANCIDAS®), imidazole, triazoles (*e.g.*, fluconazole (DIFLUCAN®)), and itraconazole (SPORANOX®)), polyene (*e.g.*, nystatin, amphotericin B colloidal dispersion (“ABCD”)(AMPHOTEC®), liposomal amphotericin B (AMBISONE®)), potassium iodide (KI), pyrimidine (*e.g.*, flucytosine (ANCOBON®)), and voriconazole (VFEND®). In certain embodiments, an effective amount of one or more antibodies of the invention are administered in combination with one or more supportive measures to a subject in need thereof to prevent, manage, treat, and/or ameliorate a fungal infection or one or more symptoms thereof. Non-limiting examples of supportive measures include humidification of the air by an ultrasonic nebulizer, aerosolized racemic epinephrine, oral dexamethasone, intravenous fluids, intubation, fever reducers (*e.g.*, ibuprofen and acetaminophen), and anti-viral or anti-bacterial therapy (*i.e.*, to prevent or treat secondary viral or bacterial infections).

**[00369]** The invention also provides methods for preventing, managing, treating and/or ameliorating a biological response to a fungal respiratory infection such as, but not limited to, elevated levels of IgE antibodies, elevated nerve growth factor (NGF) levels, mast cell proliferation, degranulation, and/or infiltration, increased proliferation and/or infiltration of B cells, and increased proliferation and/or infiltration of T cells, said methods comprising administration of an effective amount of one or more antibodies that immunospecifically bind to an IL-9 polypeptide alone or in combination with one or more other therapies.

**[00370]** In a specific embodiment, the invention provides methods for preventing, treating, managing, and/or ameliorating one or more secondary conditions or responses to a primary fungal infection, preferably a primary fungal respiratory infection, said method comprising of administering to a subject in need thereof an effective amount of one or more antibodies of the invention alone or in combination with an effective amount of other therapies (*e.g.*, other prophylactic or therapeutic agents) other than antibodies of the invention. Examples of secondary conditions or responses to a primary fungal infection, particularly primary fungal respiratory infection include, but are not limited to, asthma-like

responsiveness to mucosal stimuli, elevated total respiratory resistance, increased susceptibility to secondary viral, fungal, and fungal infections, and development of such conditions such as, but not limited to, pneumonia, croup, and febrile bronchitis.

[00371] In a specific embodiment, the invention provides methods to prevent, treat, manage, and/or ameliorate a fungal infection, preferably a fungal respiratory infection, or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™ (MedImmune, Inc., International Publication No. WO 00/78815, International Publication No. WO 02/070007 A1, dated September 12, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin AlphaV Beta3 Antagonists,” International Publication No. WO 03/075957 A1, dated September 18, 2003, entitled “The Prevention or Treatment of Cancer Using Integrin AlphaVBeta3 Antagonists in Combination With Other Agents,” U.S. Patent Pub. No. US 2002/0168360 A1, dated November 14, 2002, entitled “Methods of Preventing or Treating Inflammatory or Autoimmune Disorders by Administering Integrin  $\alpha_v\beta_3$  Antagonists in Combination With Other Prophylactic or Therapeutic Agents,” and International Publication No. WO 03/075741 A2, dated September 18, 2003, entitled, “Methods of Preventing or Treating Disorders by Administering an Integrin  $\alpha_v\beta_3$  Antagonist in Combination With an HMG-CoA Reductase Inhibitor or a Bisphosphonate,” each of which is incorporated herewith by reference in its entirety) to a subject in need thereof. In another specific embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a fungal respiratory infection or one or more symptoms thereof, said methods comprising administering to a subject in need thereof an effective amount of one or more antibodies of the invention in combination with an effective amount of siplizumab (MedImmune, Inc., International Pub. No. WO 02/069904) to a subject in need thereof. In another embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a fungal respiratory infection or one or more symptoms thereof, said methods comprising administering an effective amount of one or more antibodies of the invention in combination with an effective amount of one or more EphA2 inhibitors (*e.g.*, one or more anti-EphA2 antibodies (MedImmune, Inc.; International Publication No. WO 02/102974 A4, dated December 27, 2002, entitled “Mutant Proteins, High Potency Inhibitory Antibodies and FIMCH Crystal Structure,” International Publication No. 03/094859 A2, dated November 20, 2003, entitled “EphA2 Monoclonal Antibodies and Methods of Use Thereof,” U.S. Appn. No. 10/436,783; and U.S. Appn. No. 60/379,368, each of which is incorporated herewith by reference)) to a



subject in need thereof. In yet another embodiment, the invention provides methods of preventing, treating, managing, and/or ameliorating a fungal infection, preferably a fungal respiratory infection, or one or more symptoms thereof, said methods comprising administering an effective amount of one or more antibodies of the invention in combination with an effective amount of VITAXIN™, sipilizumab, and/or EphA2 to a subject in need thereof.

**[00372]** The invention encompasses methods for preventing the development of fungal respiratory infections in a patient expected to suffer from a fungal infection, preferably a fungal respiratory infection, or at increased risk of such an infection. Such subjects include, but are not limited to, patients with suppressed immune systems (e.g., patients organ-transplant recipients, AIDS patients, patients undergoing chemotherapy, patients with carcinoma of the esophagus with obstruction, patients with tracheobronchial fistula, patients with neurological diseases (e.g., caused by stroke, amyotrophic lateral sclerosis, multiple sclerosis, and myopathies), and patients already suffering from a respiratory condition, particularly a respiratory infection). In a specific embodiment, the patient suffers from bronchopulmonary dysplasia, congenital heart disease, cystic fibrosis, and/or acquired or congenital immunodeficiency. In another specific embodiment, the patient is an infant born prematurely, an infant, a child, an elderly human, or a human in a group home, nursing home, or some other type of institution. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating a respiratory condition or one or more symptoms thereof in patients who are susceptible to adverse reactions to conventional therapies for respiratory conditions for which no therapies are available.

**[00373]** The antibodies of the invention or combination therapies of the invention may be used as the first, second, third, fourth, or fifth therapy to prevent, manage, treat, and/or ameliorate a fungal infection, preferably a fungal respiratory infection or one or more symptom thereof. The invention also includes methods of preventing, treating, managing, and/or ameliorating a fungal infection, preferably a fungal respiratory infection or one or more symptoms thereof in a patient undergoing therapies for other disease or disorders. The invention encompasses methods of preventing, managing, treating, and/or ameliorating a fungal infection, preferably a fungal respiratory infection or one or more symptoms thereof in a patient before any adverse effects or intolerance to therapies other antibodies of the invention develops. The invention also encompasses methods of preventing, treating, managing, and/or ameliorating a fungal infection, preferably a fungal respiratory infection or a symptom thereof in refractory patients. In certain embodiments, a

patient with a fungal infection, preferably a fungal respiratory infection, is refractory to a therapy when the infection has not significantly been eradicated and/or the symptoms have not been significantly alleviated. The determination of whether a patient is refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of a treatment of infections, using art-accepted meanings of “refractory” in such a context. In various embodiments, a patient with a fungal infection, preferably a fungal respiratory infection, is refractory when fungal replication has not decreased or has increased. The invention also encompasses methods of preventing the onset or reoccurrence of fungal infections, preferably fungal respiratory infections, in patients at risk of developing such infections. The invention also encompasses methods of preventing, managing, treating, and/or ameliorating a fungal infection, preferably a fungal respiratory infection, or a symptom thereof in patients who are susceptible to adverse reactions to conventional therapies. The invention further encompasses methods for preventing, treating, managing, and/or ameliorating fungal infections, preferably fungal respiratory infections, for which no anti-fungal therapy is available.

**[00374]** The invention encompasses methods for preventing, treating, managing, and/or ameliorating a fungal infection, preferably a fungal respiratory infection, or a symptom thereof in a patient who has proven refractory to therapies other than antibodies of the invention but are no longer on these therapies. In certain embodiments, the patients being managed or treated in accordance with the methods of this invention are patients already being treated with antibiotics, anti-virals, anti-fungals, or other biological therapy/immunotherapy. Among these patients are refractory patients, patients who are too young for conventional therapies, and patients with reoccurring fungal infections despite management or treatment with existing therapies.

**[00375]** The present invention provides methods for preventing, treating, managing, and/or ameliorating a fungal infection, preferably a fungal respiratory infection, or one or more symptoms thereof as an alternative to other conventional therapies. In specific embodiments, the patient being managed or treated in accordance with the methods of the invention is refractory to other therapies or is susceptible to adverse reactions from such therapies. The patient may be a person with a suppressed immune system (*e.g.*, post-operative patients, chemotherapy patients, and patients with immunodeficiency disease), a person with impaired renal or liver function, the elderly, children, infants, infants born prematurely, persons with neuropsychiatric disorders or those who take psychotropic drugs, persons with histories of seizures, or persons on medication that would negatively interact

with conventional agents used to prevent, manage, treat, and/or ameliorate a fungal infection, preferably a fungal respiratory infection, or one or more symptoms thereof.

[00376] Fungal infection therapies and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (57th ed., 2003).

#### **5.4 COMPOSITIONS & METHODS OF ADMINISTERING ANTIBODIES**

[00377] The invention provides for the prevention, treatment, management, and/or amelioration of disorders associated with aberrant expression and/or activity of an IL-9 polypeptide, disorders associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, autoimmune disorders, inflammatory disorders, and infections (preferably, respiratory infections, or one or more symptoms thereof. In a specific embodiment, a composition comprises one or more antibodies of the invention. In another embodiment, a composition comprises one or more antibodies of the invention and one or more prophylactic or therapeutic agents, other than the antibodies of the invention, said agents known to be useful for or having been or currently used for the prevention, treatment, management, and/or amelioration of disorders associated with aberrant expression and/or activity of an IL-9 polypeptide, disorders associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, autoimmune disorders, inflammatory disorders, and infections (preferably, respiratory infections, or one or more symptoms thereof.

[00378] In one embodiment, a composition comprises one or more peptides, polypeptides, or proteins comprising a fragment of an antibody of the invention that immunospecifically binds to an IL-9 polypeptide. In another embodiment, a composition comprises one or more peptides, polypeptides, or proteins comprising a fragment of an antibody of the invention that immunospecifically binds to an IL-9 polypeptide in combination with one or more other therapies (*e.g.*, one or more prophylactic or therapeutic agents), other than a peptide, polypeptide, or protein comprising a fragment of an antibody of the invention.

[00379] In a specific embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more immunomodulatory agents. In a specific embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention,

and one or more mast cell modulators. In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more anti-angiogenic agents. In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more anti-inflammatory agents. In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more anti-cancer agents. In accordance with this embodiment, the anti-cancer agent may or may not be an immunomodulatory agent or an anti-angiogenic agent. In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more anti-viral agents. In another embodiment, a composition comprising one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, or one or more anti-bacterial agents. In another embodiment, a composition comprising one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, or one or more anti-fungal agents. In yet another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and any combination of one, two, three, or more of each of the following prophylactic or therapeutic agents: an immunomodulatory agent, a mast cell modulator, an anti-angiogenic agent, an anti-cancer agent other than an immunomodulatory agent or anti-angiogenic agent, an anti-inflammatory agent, an anti-viral agent, an anti-bacterial agent, an anti-fungal agent.

**[00380]** In a specific embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention and one or more TNF- $\alpha$  antagonists (*e.g.*, ENBREL™ and/or REMICADE®). In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and one or more integrin  $\alpha_v\beta$  antagonists. In another embodiment, a composition comprises one or more antibodies of the invention, or one or more polypeptides, peptides, or proteins comprising an antibody fragment of the invention, and VITAXIN™, sipilizumab, palivizumab, an EphA2 inhibitor, or any combination thereof. In addition to prophylactic or therapeutic agents, the compositions of the invention may also comprise a carrier.

**[00381]** The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms (*See* co-pending U.S. Provisional Appn. filed concurrently herewith (identified by Attorney Docket Number 10271-126-888) entitled “Anti-IL-9 Antibody Formulations and Uses Thereof” which is incorporated by reference herein in its entirety). In a preferred embodiment, a composition of the invention is a pharmaceutical composition. Such compositions comprise a prophylactically or therapeutically effective amount of one or more prophylactic or therapeutic agents (*e.g.*, an antibody of the invention; polypeptide, peptide, or protein comprising an antibody fragment of the invention, or other prophylactic or therapeutic agent), and a pharmaceutically acceptable carrier. Preferably, the pharmaceutical compositions are formulated to be suitable for the route of administration to a subject. In a preferred embodiment, a pharmaceutical composition of the invention is formulated in single dose vials as a sterile liquid that contains 10 mM histidine buffer at pH 6.0 and 150 mM sodium chloride. Each 1.0 mL of solution contains 100 mg of protein, 1.6 mg of histidine and 8.9 mg of sodium chloride in water for optimal stability and solubility. A more detailed description of liquid formulations containing an antibody of the invention or fragment thereof is provided in a U.S. Provisional Application to be concurrently filed herewith (identified by Attorney Docket Number 10271-126-888), entitled “Anti-IL-9 Antibody Formulations and Uses Thereof.”

**[00382]** In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions,

suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

**[00383]** Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[00384]** The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

**[00385]** Various delivery systems are known in the art and can be used to administer a prophylactic or therapeutic agent or composition of the invention to prevent, treat, manage, and/or ameliorate a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorders, an autoimmune disorders, a proliferative disorder, or a infection (preferably, a respiratory infection) or one or more symptoms thereof, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a therapy (*e.g.*, prophylactic or therapeutic agent) of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (*e.g.*, intranasal and oral routes). In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety. In one embodiment, an antibody, combination therapy, or a composition of the

invention is administered using Alkermes AIR™ pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

**[00386]** In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (*e.g.*, Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection). In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (*e.g.*, one or more prophylactic or therapeutic agents) other than an antibody of the invention to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection). In another embodiment, an effective amount of a therapy such as a mast cell modulator (*e.g.*, stem cell factor (c-kit receptor ligand) inhibitor (*e.g.*, mAb 7H6, mAb 8H7a, pAb 1337, FK506, CsA, dexamthasone, and fluconcinonide), a c-kit receptor inhibitor (*e.g.*, STI 571 (formerly known as CGP 57148B)) a mast cell protease inhibitor (*e.g.*, GW-45, GW-58, wortmannin, LY 294002, calphostin C, cytochalasin D, genistein, KT5926, staurosporine, and lactoferrin), and relaxin (“RLX”)) is administered locally to the affected area to a subject at risk of or with a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, disease or

disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection).

**[00387]** In yet another embodiment, a therapy of the invention can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald *et al.*, 1980, Surgery 88:507; Saudek *et al.*, 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., Macromol. Sci. Rev. Macromol. Chem. 23:61; see also Levy *et al.*, 1985, Science 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, J. Neurosurg. 71:105); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

**[00388]** Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, 1996, "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology



50:372-397, Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in their entirety.

**[00389]** In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered *in vivo* to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, *e.g.*, Joliot *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

**[00390]** A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral, intranasal (*e.g.*, inhalation), transdermal (*e.g.*, topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

**[00391]** If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, PA (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders,

liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (*e.g.*, preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (*e.g.*, a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

**[00392]** If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, *e.g.*, gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

**[00393]** If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts,

flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

**[00394]** The method of the invention may comprise pulmonary administration, *e.g.*, by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968, 5,985, 320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety. In a specific embodiment, an antibody of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR™ pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA).

**[00395]** The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (*e.g.*, by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (*e.g.*, in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) before use.

**[00396]** The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (*e.g.*, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (*e.g.*, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (*e.g.*, as a sparingly soluble salt).

**[00397]** The methods of the invention encompasses administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

**[00398]** Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be

dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[00399]** In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (*e.g.*, with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2°C and 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2°C and 8°C in its original container.

**[00400]** Generally, the ingredients of the compositions of the invention are derived from a subject that is the same species origin or species reactivity as recipient of such compositions. Thus, in a preferred embodiment, human or humanized antibodies are administered to a human patient for therapy or prophylaxis.

### 5.4.1 Gene Therapy

**[00401]** In a specific embodiment, nucleotide sequences comprising nucleic acids encoding an antibody of the invention or another prophylactic or therapeutic agent are administered to treat, prevent, manage, and/or ameliorate respiratory infection or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody of the invention or prophylactic or therapeutic agent that mediates a prophylactic or therapeutic effect.

**[00402]** Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel *et al.*, 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

**[00403]** In one embodiment, the method of the invention comprises administration of a composition comprising nucleic acids encoding an antibody of the invention or another prophylactic or therapeutic agent, said nucleic acids being part of an expression vector that expresses an antibody of the invention, another prophylactic or therapeutic agent, or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another embodiment, nucleic acid molecules are used in which the coding sequences of an antibody of the invention or another prophylactic or therapeutic agent and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA* 86:8932-8935; Zijlstra *et al.*, 1989, *Nature* 342:435-438). In specific embodiments, the expressed antibody of the invention or other prophylactic or therapeutic agent is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody of the invention or another prophylactic or therapeutic agent.

**[00404]** Delivery of the nucleic acids into a subject may be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

**[00405]** In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where they are expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors). In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, International Publication Nos. WO 92/06180; WO 92/22635; W092/20316; W093/14188; and WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; and Zijlstra *et al.*, 1989, Nature 342:435-438).

**[00406]** In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention, another prophylactic or therapeutic agent, or fragments thereof are used. For example, a retroviral vector can be used (see Miller *et al.*, 1993, Meth. Enzymol. 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding an antibody of the invention or another prophylactic or therapeutic agent to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen *et al.*, 1994, Biotherapy 6:291-302, which describes the use

of a retroviral vector to deliver the *mdr 1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, 1994, *J. Clin. Invest.* 93:644-651; Klein *et al.*, 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics and Devel.* 3:110-114.

**[00407]** Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics and Development* 3:499-503 present a review of adenovirus-based gene therapy. Bout *et al.*, 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, 1991, *Science* 252:431-434; Rosenfeld *et al.*, 1992, *Cell* 68:143-155; Mastrangeli *et al.*, 1993, *J. Clin. Invest.* 91:225-234; PCT Publication W094/12649; and Wang *et al.*, 1995, *Gene Therapy* 2:775-783. In a preferred embodiment, adenovirus vectors are used.

**[00408]** Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; and U.S. Patent No. 5,436,146).

**[00409]** Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

**[00410]** In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599-618; Cohen *et al.*, 1993, *Meth. Enzymol.* 217:618-644; *Clin. Pharma.*

Ther. 29:69-92 (1985)) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[00411] The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the several factors including, but not limited to, the desired effects and the patient state, and can be determined by one skilled in the art.

[00412] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, mast cells, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells (*e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.). In a preferred embodiment, the cell used for gene therapy is autologous to the subject.

[00413] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or fragment thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.*, PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

[00414] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

## **5.5 DOSAGE & FREQUENCY OF ADMINISTRATION**

[00415] The amount of a prophylactic or therapeutic agent or a composition of the invention which will be effective in the prevention, treatment, management, and/or



amelioration of a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, or an infections (preferably, a respiratory infection), or one or more symptoms thereof can be determined by standard clinical methods. The frequency and dosage will vary also according to factors specific for each patient depending on the specific therapies (*e.g.*, the specific therapeutic or prophylactic agent or agents) administered, the severity of the disorder, disease, or condition, the route of administration, as well as age, body, weight, response, and the past medical history of the patient. For example, the dosage of a prophylactic or therapeutic agent or a composition of the invention which will be effective in the treatment, prevention, management, and/or amelioration of a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, or an infections (preferably, a respiratory infection), or one or more symptoms thereof can be determined by administering the composition to an animal model such as, *e.g.*, the animal models disclosed herein or known in to those skilled in the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. Suitable regimens can be select<sup>4</sup>ed by one skilled in the art by considering such factors and by following, for example, dosages are reported in literature and recommended in the *Physician's Desk Reference* (57th ed., 2003).

**[00416]** Exemplary doses of a small molecule include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram).

**[00417]** For antibodies, proteins, polypeptides, peptides and fusion proteins encompassed by the invention, the dosage administered to a patient is typically 0.0001 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides.

Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention or fragments thereof may be reduced by enhancing uptake and tissue penetration of the antibodies by modifications such as, for example, lipidation.

**[00418]** In a specific embodiment, the dosage administered to a patient will be calculated using the patient's weight in kilograms (kg) multiplied by the dose to be administered in mg/kg. The required volume (in mL) to be given is then determined by taking the mg dose required divided by the concentration of the antibody or fragment thereof in the formulations (100 mg/mL). The final calculated required volume will be obtained by pooling the contents of as many vials as are necessary into syringe(s) to administer the drug. A maximum volume of 2.0 mL of antibody or fragment thereof in the formulations can be injected per site.

**[00419]** In a specific embodiment, the dosage of antibodies, compositions, or combination therapies of the invention administered to prevent, treat, manage, and/or ameliorate a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, or an infection (preferably, a respiratory infection), or one or more symptoms thereof in a patient is 150 µg/kg or less, preferably 125 µg/kg or less, 100 µg/kg or less, 95 µg/kg or less, 90 µg/kg or less, 85 µg/kg or less, 80 µg/kg or less, 75 µg/kg or less, 70 µg/kg or less, 65 µg/kg or less, 60 µg/kg or less, 55 µg/kg or less, 50 µg/kg or less, 45 µg/kg or less, 40 µg/kg or less, 35 µg/kg or less, 30 µg/kg or less, 25 µg/kg or less, 20 µg/kg or less, 15 µg/kg or less, 10 µg/kg or less, 5 µg/kg or less, 2.5 µg/kg or less, 2 µg/kg or less, 1.5 µg/kg or less, 1 µg/kg or less, 0.5 µg/kg or less, or 0.5 µg/kg or less of a patient's body weight. In another embodiment, the dosage of the antibodies, compositions, or combination therapies of the invention administered to prevent, treat, manage, and/or ameliorate a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more symptoms thereof in a patient is a unit dose of 0.1 mg to 20 mg, 0.1 mg to 15 mg, 0.1 mg to 12 mg, 0.1 mg to 10 mg, 0.1 mg to 8 mg, 0.1 mg to 7 mg, 0.1 mg to 5 mg, 0.1 to 2.5 mg, 0.25 mg to 20 mg, 0.25 to 15 mg, 0.25 to 12 mg, 0.25 to 10 mg, 0.25 to 8 mg, 0.25 mg to 7 mg, 0.25 mg to 5 mg, 0.5 mg to 2.5 mg, 1 mg to 20 mg, 1 mg to 15 mg, 1 mg to 12 mg, 1 mg to 10 mg, 1 mg to 8 mg, 1 mg to 7 mg, 1 mg to 5 mg, or 1 mg to 2.5 mg.

**[00420]** In certain embodiments, a subject is administered one or more doses of an effective amount of one or more antibodies, compositions, or combination therapies of the invention, wherein the an effective amount of said antibodies, compositions, or combination therapies prevents at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least 35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, or up to at least 85% of endogenous IL-9 from binding to its receptor. In certain embodiments, a subject is administered one or more doses of an effective amount of one or more or more antibodies, compositions, or combination therapies of the invention, wherein the dose of an effective amount of said antibodies, compositions, or combination therapies reduces and/or inhibits mast cell degranulation at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least 35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, at least 80 to 85%, at least 85% to 90%, at least 90% to 95%, or at least 95% to 98% relative to a control such as PBS in an *in vitro* and/or *in vivo* assay well-known in the art. In certain embodiments, a subject is administered one or more doses of an effective amount of one or more or more antibodies, compositions, or combination therapies of the invention, wherein the dose of an effective amount of said antibodies, compositions, or combination therapies reduces and/or inhibits mast cell activation at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least 35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, at least 80 to 85%, at least 85% to 90%, at least 90% to 95%, or at least 95% to 98% relative to a control such as PBS in an *in vitro* and/or *in vivo* assay well-known in the art. In certain embodiments, a subject is administered one or more doses of an effective amount of one or more or more antibodies, compositions, or combination therapies of the invention, wherein the dose of an effective amount of said antibodies, compositions, or combination therapies reduces and/or inhibits mast cell proliferation at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least 35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, at least 80 to 85%, at least 85% to 90%, at least 90% to 95%, or at least 95% to 98% relative to a control such as PBS in an *in vitro* and/or *in vivo* assay well-known in the art. In certain embodiments, a subject is administered one or more doses of an effective amount of one or more or more antibodies, compositions, or combination therapies of the invention, wherein the dose of an

effective amount of said antibodies, compositions, or combination therapies reduces and/or inhibits mast cell infiltration at least 20% to 25%, preferably at least 25% to 30%, at least 30% to 35%, at least 35% to 40%, at least 40% to 45%, at least 45% to 50%, at least 50% to 55%, at least 55% to 60%, at least 60% to 65%, at least 65% to 70%, at least 70% to 75%, at least 75% to 80%, at least 80 to 85%, at least 85% to 90%, at least 90% to 95%, or at least 95% to 98% relative to a control such as PBS in an *in vitro* and/or *in vivo* assay well-known in the art.

**[00421]** In other embodiments, a subject is administered one or more doses of an effective amount of one or more antibodies of the invention, wherein the dose of an effective amount achieves a serum titer of at least 0.1 µg/ml, at least 0.5 µg/ml, at least 1 µg/ml, at least 2 µg/ml, at least 5 µg/ml, at least 6 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, at least 25 µg/ml, at least 50 µg/ml, at least 100 µg/ml, at least 125 µg/ml, at least 150 µg/ml, at least 175 µg/ml, at least 200 µg/ml, at least 225 µg/ml, at least 250 µg/ml, at least 275 µg/ml, at least 300 µg/ml, at least 325 µg/ml, at least 350 µg/ml, at least 375 µg/ml, or at least 400 µg/ml of the antibodies of the invention. In yet other embodiments, a subject is administered a dose of an effective amount of one or more antibodies of the invention to achieve a serum titer of at least 0.1 µg/ml, at least 0.5 µg/ml, at least 1 µg/ml, at least, 2 µg/ml, at least 5 µg/ml, at least 6 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, at least 25 µg/ml, at least 50 µg/ml, at least 100 µg/ml, at least 125 µg/ml, at least 150 µg/ml, at least 175 µg/ml, at least 200 µg/ml, at least 225 µg/ml, at least 250 µg/ml, at least 275 µg/ml, at least 300 µg/ml, at least 325 µg/ml, at least 350 µg/ml, at least 375 µg/ml, or at least 400 µg/ml of the antibodies and a subsequent dose of an effective amount of one or more antibodies of the invention is administered to maintain a serum titer of at least 0.1 µg/ml, 0.5 µg/ml, 1 µg/ml, at least, 2 µg/ml, at least 5 µg/ml, at least 6 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, at least 25 µg/ml, at least 50 µg/ml, at least 100 µg/ml, at least 125 µg/ml, at least 150 µg/ml, at least 175 µg/ml, at least 200 µg/ml, at least 225 µg/ml, at least 250 µg/ml, at least 275 µg/ml, at least 300 µg/ml, at least 325 µg/ml, at least 350 µg/ml, at least 375 µg/ml, or at least 400 µg/ml. In accordance with these embodiments, a subject may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more subsequent doses.

**[00422]** In a specific embodiment, the invention provides methods of preventing, treating, managing, or treating a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease,

or an infection (preferably, a respiratory infection) or one or more symptoms thereof, said method comprising administering to a subject in need thereof a dose of at least 10  $\mu\text{g}$ , preferably at least 15  $\mu\text{g}$ , at least 20  $\mu\text{g}$ , at least 25  $\mu\text{g}$ , at least 30  $\mu\text{g}$ , at least 35  $\mu\text{g}$ , at least 40  $\mu\text{g}$ , at least 45  $\mu\text{g}$ , at least 50  $\mu\text{g}$ , at least 55  $\mu\text{g}$ , at least 60  $\mu\text{g}$ , at least 65  $\mu\text{g}$ , at least 70  $\mu\text{g}$ , at least 75  $\mu\text{g}$ , at least 80  $\mu\text{g}$ , at least 85  $\mu\text{g}$ , at least 90  $\mu\text{g}$ , at least 95  $\mu\text{g}$ , at least 100  $\mu\text{g}$ , at least 105  $\mu\text{g}$ , at least 110  $\mu\text{g}$ , at least 115  $\mu\text{g}$ , or at least 120  $\mu\text{g}$  of one or more antibodies, combination therapies, or compositions of the invention. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof, said methods comprising administering to a subject in need thereof a dose of at least 10  $\mu\text{g}$ , preferably at least 15  $\mu\text{g}$ , at least 20  $\mu\text{g}$ , at least 25  $\mu\text{g}$ , at least 30  $\mu\text{g}$ , at least 35  $\mu\text{g}$ , at least 40  $\mu\text{g}$ , at least 45  $\mu\text{g}$ , at least 50  $\mu\text{g}$ , at least 55  $\mu\text{g}$ , at least 60  $\mu\text{g}$ , at least 65  $\mu\text{g}$ , at least 70  $\mu\text{g}$ , at least 75  $\mu\text{g}$ , at least 80  $\mu\text{g}$ , at least 85  $\mu\text{g}$ , at least 90  $\mu\text{g}$ , at least 95  $\mu\text{g}$ , at least 100  $\mu\text{g}$ , at least 105  $\mu\text{g}$ , at least 110  $\mu\text{g}$ , at least 115  $\mu\text{g}$ , or at least 120  $\mu\text{g}$  of one or more antibodies, combination therapies, or compositions of the invention once every 3 days, preferably, once every 4 days, once every 5 days, once every 6 days, once every 7 days, once every 8 days, once every 10 days, once every two weeks, once every three weeks, or once a month.

**[00423]** The present invention provides methods of preventing, treating, managing, or preventing a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof, said method comprising: (a) administering to a subject in need thereof one or more doses of a prophylactically or therapeutically effective amount of one or more antibodies, combination therapies, or compositions of the invention; and (b) monitoring the plasma level/concentration of the said administered antibody or antibodies in said subject after administration of a certain number of doses of the said antibody or antibodies. Moreover, preferably, said certain number of doses is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 doses of a prophylactically or therapeutically effective amount one or more antibodies, compositions, or combination therapies of the invention.

**[00424]** In a specific embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof, said method comprising: (a) administering to a subject in need thereof a dose of at least 10 µg (preferably at least 15 µg, at least 20 µg, at least 25 µg, at least 30 µg, at least 35 µg, at least 40 µg, at least 45 µg, at least 50 µg, at least 55 µg, at least 60 µg, at least 65 µg, at least 70 µg, at least 75 µg, at least 80 µg, at least 85 µg, at least 90 µg, at least 95 µg, or at least 100 µg) of one or more antibodies of the invention; and (b) administering one or more subsequent doses to said subject when the plasma level of the antibody or antibodies administered in said subject is less than 0.1 µg/ml, preferably less than 0.25 µg/ml, less than 0.5 µg/ml, less than 0.75 µg/ml, or less than 1 µg/ml. In another embodiment, the invention provides a method of preventing, treating, managing, and/or ameliorating a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof, said method comprising: (a) administering to a subject in need thereof one or more doses of at least 10 µg (preferably at least 15 µg, at least 20 µg, at least 25 µg, at least 30 µg, at least 35 µg, at least 40 µg, at least 45 µg, at least 50 µg, at least 55 µg, at least 60 µg, at least 65 µg, at least 70 µg, at least 75 µg, at least 80 µg, at least 85 µg, at least 90 µg, at least 95 µg, or at least 100 µg) of one or more antibodies of the invention; (b) monitoring the plasma level of the administered antibody or antibodies of the invention in said subject after the administration of a certain number of doses; and (c) administering a subsequent dose of the antibody or antibodies of the invention when the plasma level of the administered antibody or antibodies in said subject is less than 0.1 µg/ml, preferably less than 0.25 µg/ml, less than 0.5 µg/ml, less than 0.75 µg/ml, or less than 1 µg/ml. Preferably, said certain number of doses is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 doses of an effective amount of one or more antibodies of the invention.

**[00425]** Therapies (*e.g.*, prophylactic or therapeutic agents), other than antibodies of the invention, which have been or are currently being used to prevent, treat, manage, and/or ameliorate a disease or disorder associated with or characterized by aberrant expression

and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof can be administered in combination with one or more antibodies of the invention according to the methods of the invention to treat, manage, prevent, and/or ameliorate a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof. Preferably, the dosages of prophylactic or therapeutic agents used in combination therapies of the invention are lower than those which have been or are currently being used to prevent, treat, manage, and/or ameliorate a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof. The recommended dosages of agents currently used for the prevention, treatment, management, or amelioration of a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disease, an inflammatory disease, a proliferative disease, or an infection (preferably, a respiratory infection) or one or more symptoms thereof can be obtained from any reference in the art including, but not limited to, Hardman *et al.*, eds., 2001, Goodman & Gilman's The Pharmacological Basis Of Basis Of Therapeutics, 10th ed., Mc-Graw-Hill, New York; Physician's Desk Reference (PDR) 57th ed., 2003, Medical Economics Co., Inc., Montvale, NJ, which are incorporated herein by reference in its entirety.

**[00426]** In various embodiments, the therapies (*e.g.*, prophylactic or therapeutic agents) are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about

12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart. In preferred embodiments, two or more therapies are administered within the same patent visit.

**[00427]** In certain embodiments, one or more antibodies of the invention and one or more other therapies (*e.g.*, prophylactic or therapeutic agents) are cyclically administered. Cycling therapy involves the administration of a first therapy (*e.g.*, a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (*e.g.*, a second prophylactic or therapeutic agent) for a period of time, optionally, followed by the administration of a third therapy (*e.g.*, prophylactic or therapeutic agent) for a period of time and so forth, and repeating this sequential administration, *i.e.*, the cycle in order to reduce the development of resistance to one of the therapies, to avoid or reduce the side effects of one of the therapies, and/or to improve the efficacy of the therapies.

**[00428]** In certain embodiments, the administration of the same antibodies of the invention may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In other embodiments, the administration of the same therapy (*e.g.*, prophylactic or therapeutic agent) other than an antibody of the invention may be repeated and the administration may be separated by at least at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months.

## **5.6 BIOLOGICAL ASSAYS**

### **5.6.1 Immunospecificity of Antibodies of the Invention**

**[00429]** Antibodies of the present invention or fragments thereof may be characterized in a variety of ways well-known to one of skill in the art. In particular, antibodies of the invention or fragments thereof may be assayed for the ability to immunospecifically bind to an IL-9 polypeptide. Such an assay may be performed in solution (*e.g.*, Houghten, 1992, *Bio/Techniques* 13:412-421), on beads (Lam, 1991, *Nature* 354:82-84), on chips (Fodor, 1993, *Nature* 364:555-556), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or on phage (Scott and Smith, 1990, *Science* 249:386-390; Cwirla *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici, 1991, *J. Mol. Biol.* 222:301-310) (each of these references is incorporated herein in its entirety by reference). Antibodies or fragments thereof that have been identified to



immunospecifically bind to an IL-9 polypeptide can then be assayed for their specificity and affinity for an IL-9 polypeptide.

**[00430]** The antibodies of the invention or fragments thereof may be assayed for immunospecific binding to an IL-9 polypeptide and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel *et al.*, eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

**[00431]** Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel *et al.*, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

**[00432]** Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, incubating the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), incubating the membrane with primary

antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, incubating the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

[00433] ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. In a preferred embodiment, an ELISA may be performed by coating a high binding 96-well microtiter plate (Costar) with  $2\mu\text{g/ml}$  of rhu-IL-9 in PBS overnight. Following three washes with PBS, the plate is incubated with three-fold serial dilutions of Fab at  $25^{\circ}\text{C}$  for 1 hour. Following another three washes of PBS,  $1\mu\text{g/ml}$  anti-human kappa-alkaline phosphatase-conjugate is added and the plate is incubated for 1 hour at  $25^{\circ}\text{C}$ . Following three washes with PBST, the alkaline phosphatase activity is determined in  $50\mu\text{l/AMP/PPMP}$  substrate. The reactions are stopped and the absorbance at 560 nm is determined with a VMAX microplate reader. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[00434] The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing

amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention or a fragment thereof for an IL-9 polypeptide and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, an IL-9 polypeptide is incubated with an antibody of the present invention conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

**[00435]** In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies of the invention to an IL-9 polypeptide. BIAcore kinetic analysis comprises analyzing the binding and dissociation of an IL-9 polypeptide from chips with immobilized antibodies of the invention on their surface. A typical BIAcore kinetic study involves the injection of 250  $\mu\text{L}$  of an antibody reagent (mAb, Fab) at varying concentration in HBS buffer containing 0.005% Tween-20 over a sensor chip surface, onto which has been immobilized the antigen. The flow rate is maintained constant at 75  $\mu\text{L}/\text{min}$ . Dissociation data is collected for 15 min. or longer as necessary. Following each injection/dissociation cycle, the bound mAb is removed from the antigen surface using brief, 1 min. pulses of dilute acid, typically 10-100 mM HCl, though other regenerants are employed as the circumstances warrant. More specifically, for measurement of the rates of association,  $k_{\text{on}}$ , and dissociation,  $k_{\text{off}}$ , the antigen is directly immobilized onto the sensor chip surface through the use of standard amine coupling chemistries, namely the EDC/NHS method (EDC= N-diethylaminopropyl)-carbodiimide). Briefly, a 5-100 nM solution of the antigen in 10mM NaOAc, pH4 or pH5 is prepared and passed over the EDC/NHS-activated surface until approximately 30-50 RU's worth of antigen are immobilized. Following this, the unreacted active esters are "capped" off with an injection of 1M Et-NH<sub>2</sub>. A blank surface, containing no antigen, is prepared under identical immobilization conditions for reference purposes. Once an appropriate surface has been prepared, a suitable dilution series of each one of the antibody reagents is prepared in HBS/Tween-20, and passed over both the antigen and reference cell surfaces, which are connected in series. The range of antibody concentrations that are prepared varies, depending on what the equilibrium binding constant,  $K_D$ , is estimated to be. As described above, the bound antibody is removed after each injection/dissociation cycle using an appropriate regenerant.

**[00436]** The antibodies of the invention or fragments thereof can also be assayed for their ability to inhibit the binding of IL-9 to its host cell receptor using techniques known to those of skill in the art. For example, cells expressing IL-9 receptor can be contacted with IL-9 in the presence or absence of an antibody or fragment thereof and the ability of the

antibody or fragment thereof to inhibit IL-9's binding can be measured by, for example, flow cytometry or a scintillation assay. IL-9 or the antibody or antibody fragment can be labeled with a detectable compound such as a radioactive label (*e.g.*,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^{125}\text{I}$ ) or a fluorescent label (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine) to enable detection of an interaction between IL-9 and its host cell receptor. Alternatively, the ability of antibodies or fragments thereof to inhibit IL-9 from binding to its receptor can be determined in cell-free assays. For example, an IL-9 polypeptide can be contacted with an antibody or fragment thereof and the ability of the antibody or antibody fragment to inhibit the IL-9 polypeptide from binding to its host cell receptor can be determined. Preferably, the antibody or the antibody fragment is immobilized on a solid support and an IL-9 polypeptide is labeled with a detectable compound. Alternatively, an IL-9 polypeptide is immobilized on a solid support and the antibody or fragment thereof is labeled with a detectable compound. An IL-9 may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. Further, an IL-9 polypeptide may be a fusion protein comprising IL-9, a derivative, analog or fragment thereof and a domain such as glutathione-S-transferase. Alternatively, an IL-9 polypeptide can be biotinylated using techniques well known to those of skill in the art (*e.g.*, biotinylation kit, Pierce Chemicals; Rockford, IL).

**[00437]** The ability of antibodies or fragments of the invention to inhibit IL-9 from binding to its host cell receptor may be measured by cell proliferation assays. For example, the murine TS1-RA3 T cell line expressing both human and murine IL-9 $\alpha$  may be grown continuously in growth medium (DMEM) containing rhuIL-9 (25 ng/ml, R & D Systems). Upon withdrawal of rhuIL-9, TS1-RA3 undergoes cell death in 18-24 hours. TS1-RA3 cells grown in RPMI 1640 (ATCC) medium supplemented with 10% FBS and 25 ng/ml rHu-IL9. Prior to the assay, the cells are washed with media containing no IL-9 and resuspended at  $5 \times 10^5$  cells/ml in media containing 2 ng/ml rhuIL-9. The cells are distributed into a black clear bottom non-binding 96-well microtiter plate (100  $\mu\text{l}$  cells/well) and 100  $\mu\text{l}$  of serially diluted variant Fabs is then added to the plate. The plate is incubated at 72 hours at  $37^\circ\text{C}$ , 5%  $\text{CO}_2$ . 20  $\mu\text{l}$ /well of Alamar blue® is added, and the cells are incubated for an additional 4-5 hours. Cell metabolism is quantitated using a fluorimeter with excitation at 555 nm and emission at 590 nm. The ability of antibodies or fragments of the invention to inhibit IL-9 from binding to its host cell receptor may be measured may also be measured by a cell binding assay, such as an IL-9 binding ELISA assay. For example, each well of a 96-well ELISA plate is coated with 100  $\mu\text{L}$  of IL-9 antibodies or

antibody fragments of the invention overnight at 2 to 8 °C. The plate is washed three times with PBS/0.5% Tween-20 buffer, and is blocked for 1 hour at ambient temperature with PBS/0.1% Tween-20 buffer, 1% (w/v) BSA. After washing the plate, 100 µL of a Reference Standard, samples and controls are loaded onto the assay plate and incubated at ambient temperature for 1 hour. After washing, 100 µL of horseradish peroxidase-labeled (HRP) goat anti-human IgG at a 1:15,000 dilution is added to the assay plate. Following the one-hour incubation, the plate is washed and 100 µL/well of 3, 3',5,5'-tetramethylbenzidine (TMB) substrate is added to the plate and incubated at ambient temperature in the dark for 10 minutes. The enzymatic reaction is stopped by the addition of 50 µL/well of 2N sulfuric acid. The absorbance at 450 nm is measured using a microplate reader. Samples are dispositioned as pass/fail based on the parallelism of the sample curve to the Reference Standard curve, and the ED<sub>50</sub> value of the sample falling in the range of 3.91-31.91 ng/mL.

### **5.6.2 In Vitro Studies**

**[00438]** The antibodies, compositions, or combination therapies of the invention can be tested *in vitro* and/or *in vivo* for their ability to modulate the biological activity of immune cells (*e.g.*, T cells, neutrophils, and mast cells), endothelial cells, and epithelial cells. The ability of an antibody, composition, or combination therapy of the invention to modulate the biological activity of immune cells (*e.g.*, T cells, B cells, mast cells, macrophages, neutrophils, and eosinophils), endothelial cells, and epithelial cells can be assessed by: detecting the expression of antigens (*e.g.*, activation of genes by IL-9, such as, but not limited to, mucin genes (*e.g.*, MUC2, MUC5AC, MUC5B, and MUC6) and genes involved in lymphocyte activation (*e.g.*, Lgamma-6A/E)); detecting the proliferation of immune cells, endothelial cells and/or epithelial cells; detecting the activation of signaling molecules (*e.g.*, the phosphorylation of Stat2, the phosphorylation of JAK3, or the phosphorylation of the IL-9R); detecting the effector function of immune cells (*e.g.*, T cells, B cells, mast cells, macrophages, neutrophils, and eosinophils), endothelial cells, and/or epithelial cells; or detecting the differentiation of immune cells, endothelial cells, and/or epithelial cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by 3H-thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, immunohistochemistry radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich"

immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs). Mast cell degranulation can be assayed, for example by measuring serotonin (5-HT) release or histamine release with high-performance liquid chromatography (see, *e.g.*, Taylor *et al.* 1995 Immunology 86(3): 427-433 and Kurosawa *et al.*, 1998 Clin Exp Allergy 28(8): 1007-1012).

**[00439]** The antibodies, compositions, or combination therapies of the invention are preferably tested *in vitro* and then *in vivo* for the desired therapeutic or prophylactic activity prior to use in humans. For example, assays which can be used to determine whether administration of a specific pharmaceutical composition is indicated include cell culture assays in which a patient tissue sample is grown in culture and exposed to, or otherwise contacted with, a pharmaceutical composition, and the effect of such composition upon the tissue sample is observed. The tissue sample can be obtained by biopsy from the patient. This test allows the identification of the therapeutically most effective therapy (*e.g.*, prophylactic or therapeutic agent) for each individual patient. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection) to determine if a pharmaceutical composition of the invention has a desired effect upon such cell types.

**[00440]** The effect of an antibody, a composition, or a combination therapy of the invention on peripheral blood lymphocyte counts can be monitored/assessed using standard techniques known to one of skill in the art. Peripheral blood lymphocytes counts in a subject can be determined by, *e.g.*, obtaining a sample of peripheral blood from said subject, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, Ficoll-Hypaque (Pharmacia) gradient centrifugation, and counting the lymphocytes using trypan blue. Peripheral blood T-cell counts in subject can be determined by, *e.g.*, separating the lymphocytes from other components of peripheral blood such as plasma using, *e.g.*, a use of Ficoll-Hypaque (Pharmacia) gradient centrifugation, labeling the T-cells with an antibody directed to a T-cell antigen which is conjugated to FITC or phycoerythrin, and measuring the number of T-cells by FACS.

**[00441]** The methods of the invention for treating, managing, preventing, and/or ameliorating a viral respiratory infection or one or more symptoms thereof can be tested for their ability to inhibit viral replication or reduce viral load in *in vitro* assays. For example, viral replication can be assayed by a plaque assay such as described, *e.g.*, by Johnson *et al.*, 1997, Journal of Infectious Diseases 176:1215-1224 176:1215-1224. The antibodies, compositions, or combination therapies administered according to the methods of the invention can also be assayed for their ability to inhibit or downregulate the expression of viral polypeptides. Techniques known to those of skill in the art, including, but not limited to, western blot analysis, northern blot analysis, and RT-PCR can be used to measure the expression of viral polypeptides.

**[00442]** The methods of the invention for preventing, treating, managing, and/or ameliorating a respiratory infection or one or more symptoms thereof can be tested for activity against bacteria causing respiratory infections in *in vitro* assays well-known in the art. *In vitro* assays known in the art can also be used to test the existence or development of resistance of bacteria to a therapy (*e.g.*, an antibody of the invention, other prophylactic or therapeutic agent, a combination thereof, or a composition thereof) of the invention. Such *in vitro* assays are described in Gales *et al.*, 2002, Diag. Microbiol. Infect. Dis. 44(3):301-311; Hicks *et al.*, 2002, Clin. Microbiol. Infect. 8(11): 753-757; and Nicholson *et al.*, 2002, Diagn. Microbiol. Infect. Dis. 44(1): 101-107.

**[00443]** The therapies (*e.g.*, an antibody of the invention alone or in combination with prophylactic or therapeutic agents, other than antibodies of the invention) of the invention for treating, managing, preventing, and/or ameliorating a respiratory infection or one or more symptoms thereof can be tested for anti-fungal activity against different species of fungus. Any of the standard anti-fungal assays well-known in the art can be used to assess the anti-fungal activity of a therapy. The anti-fungal effect on different species of fungus can be tested. The tests recommended by the National Committee for Clinical Laboratories (NCCLS) (*See* National Committee for Clinical Laboratories Standards. 1995, Proposed Standard M27T. Villanova, Pa., all of which is incorporated herein by reference in its entirety) and other methods known to those skilled in the art (Pfaller *et al.*, 1993, *Infectious Dis. Clin. N. Am.* 7: 435-444) can be used to assess the anti-fungal effect of a therapy. The antifungal properties of a therapy may also be determined from a fungal lysis assay, as well as by other methods, including, *inter alia*, growth inhibition assays, fluorescence-based fungal viability assays, flow cytometry analyses, and other standard assays known to those skilled in the art.

**[00444]** For example, the anti-fungal activity of a therapy can be tested using macrodilution methods and/or microdilution methods using protocols well-known to those skilled in the art (*see, e.g.,* Clancy *et al.*, 1997 *Journal of Clinical Microbiology*, 35(11): 2878-82; Ryder *et al.*, 1998, *Antimicrobial Agents and Chemotherapy*, 42(5): 1057-61; U.S. 5,521,153; U.S. 5,883,120, U.S. 5,521,169, all of which are incorporated by reference in their entirety). Briefly, a fungal strain is cultured in an appropriate liquid media, and grown at an appropriate temperature, depending on the particular fungal strain used for a determined amount of time, which is also depends on the particular fungal strain used. An inoculum is then prepared photometrically and the turbidity of the suspension is matched to that of a standard, *e.g.,* a McFarland standard. The effect of a therapy on the turbidity of the inoculum is determined visually or spectrophotometrically. The minimal inhibitory concentration ("MIC") of the therapy is determined, which is defined as the lowest concentration of the lead compound which prevents visible growth of an inoculum as measured by determining the culture turbidity.

**[00445]** The anti-fungal activity of a therapy can also be determined utilizing colorimetric based assays well-known to one of skill in the art. One exemplary colorimetric assay that can be used to assess the anti-fungal activity of a therapy is described by Pfaller *et al.* (1994, *Journal of Clinical Microbiology*, 32(8): 1993-6, which is incorporated herein by reference in its entirety; also *see* Tiballi *et al.*, 1995, *Journal of Clinical Microbiology*, 33(4): 915-7). This assay employs a colorimetric endpoint using an oxidation-reduction indicator (Alamar Biosciences, Inc., Sacramento CA).

**[00446]** The anti-fungal activity of a therapy can also be determined utilizing photometric assays well-known to one of skill in the art (*see, e.g.,* Clancy *et al.*, 1997 *Journal of Clinical Microbiology*, 35(11): 2878-82; Jahn *et al.*, 1995, *Journal of Clinical Microbiology*, 33(3): 661-667, each of which is incorporated herein by reference in its entirety). This photometric assay is based on quantifying mitochondrial respiration by viable fungi through the reduction of 3-(4,5-dimethyl-2thiazolyl)-2,5,-diphenyl-2H-tetrazolium bromide (MTT) to formazan. MIC's determined by this assay are defined as the highest concentration of the test therapy associated with the first precipitous drop in optical density. In some embodiments, the therapy is assayed for anti-fungal activity using macrodilution, microdilution and MTT assays in parallel.

**[00447]** Further, any *in vitro* assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of an antibody, a composition, a combination therapy disclosed herein for a respiratory infection or one or more symptoms thereof.



### 5.6.3 In Vivo Assays

**[00448]** The antibodies, compositions, or combination therapies of the invention can be tested in suitable animal model systems prior to use in humans. Such animal model systems include, but are not limited to, rats, mice, chicken, cows, monkeys, pigs, dogs, rabbits, etc. Any animal system well-known in the art may be used. Several aspects of the procedure may vary; said aspects include, but are not limited to, the temporal regime of administering the therapies (*e.g.*, prophylactic and/or therapeutic agents), whether such therapies are administered separately or as an admixture, and the frequency of administration of the therapies.

**[00449]** Animal models for autoimmune disorders can also be used to assess the efficacy of an antibody, a composition, or a combination therapy of the invention. Animal models for autoimmune disorders such as type 1 diabetes, thyroid autoimmunity, systemic lupus erythematosus, and glomerulonephritis have been developed (Flanders *et al.*, 1999, *Autoimmunity* 29:235-246; Krogh *et al.*, 1999, *Biochimie* 81:511-515; Foster, 1999, *Semin. Nephrol.* 19:12-24).

**[00450]** Efficacy in preventing, treating, managing, and/or ameliorating an autoimmune disorder may be demonstrated, *e.g.*, by detecting the ability of an antibody, a composition, or a combination therapy of the invention to reduce one or more symptoms of the autoimmune disorder, to reduce mean absolute lymphocyte counts, to decrease T cell activation, to decrease T cell proliferation, to reduce cytokine production, or to modulate one or more particular cytokine profiles. Efficacy in preventing or treating psoriasis may be demonstrated, *e.g.*, by detecting the ability of an antibody, fragment thereof, or composition of the invention to reduce one or more symptoms of psoriasis, to reduce mean absolute lymphocyte counts, to reduce cytokine production, to modulate one or more particular cytokine profiles, to decrease scaling, to decrease erythema, to decrease plaque elevation, to decrease T cell activation in the dermis or epidermis of an affected area, to decrease T cell infiltration to the dermis or epidermis of an affected area, to reduce PASI, to improve the physician's global assessment score, or to improve quality of life.

**[00451]** Animal models for cancer can be used to assess the efficacy of an antibody, a composition, or a combination therapy of the invention. Examples of animal models for lung cancer include, but are not limited to, lung cancer animal models described by Zhang & Roth (1994, *In Vivo* 8(5):755-69) and a transgenic mouse model with disrupted p53 function (see, *e.g.*, Morris *et al.*, 1998, *J La State Med Soc* 150(4):179-85). An example of an animal model for breast cancer includes, but is not limited to, a transgenic mouse that overexpresses cyclin D1 (see, *e.g.*, Hosokawa *et al.*, 2001, *Transgenic Res* 10(5):471-8).

An example of an animal model for colon cancer includes, but is not limited to, a TCR b and p53 double knockout mouse (see, *e.g.*, Kado *et al.*, 2001, *Cancer Res* 61(6):2395-8). Examples of animal models for pancreatic cancer include, but are not limited to, a metastatic model of Panc02 murine pancreatic adenocarcinoma (see, *e.g.*, Wang *et al.*, 2001, *Int J Pancreatol* 29(1):37-46) and nu-nu mice generated in subcutaneous pancreatic tumours (see, *e.g.*, Ghaneh *et al.*, 2001, *Gene Ther* 8(3):199-208). Examples of animal models for non-Hodgkin's lymphoma include, but are not limited to, a severe combined immunodeficiency ("SCID") mouse (see, *e.g.*, Bryant *et al.*, 2000, *Lab Invest* 80(4):553-73) and an IgHmu-HOX11 transgenic mouse (see, *e.g.*, Hough *et al.*, 1998, *Proc Natl Acad Sci USA* 95(23):13853-8). An example of an animal model for esophageal cancer includes, but is not limited to, a mouse transgenic for the human papillomavirus type 16 E7 oncogene (see, *e.g.*, Herber *et al.*, 1996, *J Virol* 70(3):1873-81). Examples of animal models for colorectal carcinomas include, but are not limited to, Apc mouse models (see, *e.g.*, Fodde & Smits, 2001, *Trends Mol Med* 7(8):369-73 and Kuraguchi *et al.*, 2000, *Oncogene* 19(50):5755-63).

**[00452]** The anti-inflammatory activity of an antibody, a composition, or a combination therapy of the invention can be determined by using various experimental animal models of inflammatory arthritis known in the art and described in Crofford L.J. and Wilder R.L., "Arthritis and Autoimmunity in Animals," in *Arthritis and Allied Conditions: A Textbook of Rheumatology*, McCarty (eds.), Chapter 30 (Lee and Febiger, 1993). Experimental and spontaneous animal models of inflammatory arthritis and autoimmune rheumatic diseases can also be used to assess the anti-inflammatory activity of the antibodies, compositions, or combination therapies of invention.

**[00453]** The anti-inflammatory activity of an antibody, a composition, or a combination therapy of invention can also be assessed by measuring the inhibition of carrageenan-induced paw edema in the rat, using a modification of the method described in Winter C. A. *et al.*, "Carrageenan-Induced Edema in Hind Paw of the Rat as an Assay for Anti-inflammatory Drugs" *Proc. Soc. Exp. Biol Med.* 111, 544-547, (1962). This assay has been used as a primary *in vivo* screen for the anti-inflammatory activity of most NSAIDs, and is considered predictive of human efficacy. The anti-inflammatory activity of the test therapies (*e.g.*, prophylactic or therapeutic agents) is expressed as the percent inhibition of the increase in hind paw weight of the test group relative to the vehicle dosed control group.

**[00454]** In a specific embodiment of the invention where the experimental animal model used is adjuvant-induced arthritis rat model, body weight can be measured relative to

a control group to determine the anti-inflammatory activity of an antibody, a composition, a combination therapy of the invention.

**[00455]** Animal models for allergies and asthma are known in the art, such as constant-flow inflation with end-inspiratory occlusion described in Ewart *et al.*, 1995 J Appl Physiol 79(2):560-566 and other assays described in, *e.g.*, Komai *et al.*, 2003 Br J Pharmacol 138(5): 912-920; Kenyon *et al.*, 2003 Toxicol Appl Pharmacol 186(2): 90-100; Path *et al.*, 2002 Am J Resp & Critical Care Med 166(6): 818-826; Martins *et al.*, 1990 Crit Care Med 19:515-519; Nicolaides *et al.*, 1997 Proc Natl Acad Sci USA 94:13175-13180; McLane *et al.*, 1998 19:713-720; and Temann *et al.*, 1998 J Exp Med 188(7): 1307-1320. For example, the murine adoptive transfer model is an animal model used to assess the efficacy an antibody, a composition, or a combination therapy of the invention for the prevention, treatment, management, and/or asthma include. In the murine adoptive transfer model, aeroallergen provocation of TH1 or TH2 recipient mice results in TH effector cell migration to the airways and is associated with an intense neutrophilic (TH1) and eosinophilic (TH2) lung mucosal inflammatory response (Cohn *et al.*, 1997, J. Exp. Med. 186:1737-1747). Airway hypersensitivity can be induced in mice by ovalbumin (Tomkinson *et al.*, 2001, J. Immunol. 166:5792-5800) or Schistosoma mansoni egg antigen (Tesciuba *et al.*, 2001, J. Immunol. 167:1996-2003).

**[00456]** Efficacy in preventing or treating an inflammatory disorder may be demonstrated, *e.g.*, by detecting the ability of an antibody, a composition, or a combination therapy of the invention to reduce one or more symptoms of the inflammatory disorder, to decrease T cell activation, to decrease T cell proliferation, to modulate one or more cytokine profiles, to reduce cytokine production, to reduce inflammation of a joint, organ or tissue or to improve quality of life.

**[00457]** Changes in inflammatory disease activity may also be assessed through tender and swollen joint counts, patient and physician global scores for pain and disease activity, and the ESR/CRP. Progression of structural joint damage may be assessed by quantitative scoring of X-rays of hands, wrists, and feet (Sharp method). Changes in functional status in humans with inflammatory disorders may be evaluated using the Health Assessment Questionnaire (HAQ), and quality of life changes are assessed with the SF.

**[00458]** The efficacy of an antibody, a composition, or a combination therapy of the invention in preventing, treating, managing, and/or ameliorating Type I allergic reaction may be assessed by its ability to induce anti-IgE antibodies that inhibit IgE from binding to its receptor on mast cells or basophils *in vitro*. IgE levels can be assayed by immunoassays,

gel electrophoresis followed by visualization, radioimmunosorbent test (RIST), radioallergosorbent test (RAST), or any other method known to those skilled in the art.

**[00459]** Animal models for viral infections can also be used to assess the efficacy of an antibody, a composition, or a combination therapy of the invention. Animal models for viral infections such as EBV-associated diseases, gammaherpesviruses, infectious mononucleosis, simian immunodeficiency virus (“SIV”), Borna disease virus infection, hepatitis, varicella virus infection, viral pneumonitis, Epstein-Barr virus pathogenesis, feline immunodeficiency virus (“FIV”), HTLV type 1 infection, human rotaviruses, and genital herpes have been developed (see, *e.g.*, Hayashi *et al.*, 2002, *Histol Histopathol* 17(4):1293-310; Arico *et al.*, 2002, *J Interferon Cytokine Res* 22(11):1081-8; Flano *et al.*, 2002, *Immunol Res* 25(3):201-17; Sauermann, 2001, *Curr Mol Med* 1(4):515-22; Pletnikov *et al.*, 2002, *Front Biosci* 7:d593-607; Engler *et al.*, 2001, *Mol Immunol* 38(6):457-65; White *et al.*, 2001, *Brain Pathol* 11(4):475-9; Davis & Matalon, 2001, *News Physiol Sci* 16:185-90; Wang, 2001, *Curr Top Microbiol Immunol*. 258:201-19; Phillips *et al.*, 2000, *J Psychopharmacol*. 14(3):244-50; Kazanji, 2000, *AIDS Res Hum Retroviruses*. 16(16):1741-6; Saif *et al.*, 1996, *Arch Virol Suppl*. 12:153-61; and Hsiung *et al.*, 1984, *Rev Infect Dis*. 6(1):33-50).

**[00460]** Animal models for viral respiratory infections such as, but not limited to, PIV (see, *e.g.*, Shephard *et al.*, 2003 *Res Vet Sci* 74(2): 187-190; Ottolini *et al.*, 2002 *J Infect Dis* 186(12): 1713-1717), RSV (see, *e.g.*, Culley *et al.*, 2002 *J Exp Med* 196(10): 1381-1386; and Curtis *et al.*, 2002 *Exp Biol Med* 227(9): 799-802). In a specific embodiment, cotton rats are administered an antibody of the invention, a composition, or a combination therapy according to the methods of the invention, challenged with  $10^5$  pfu of RSV, and four or more days later the rats are sacrificed and RSV titer and anti-RSV antibody serum titer is determined. Accordingly, a dosage that results in a 2 log decrease or a 99% reduction in RSV titer in the cotton rat challenged with  $10^5$  pfu of RSV relative to the cotton rat challenged with  $10^5$  pfu of RSV but not administered the formulation is the dosage of the formulation that can be administered to a human for the treatment, prevention or amelioration of one or more symptoms associated with RSV infection. Further, in accordance with this embodiment, the tissues (*e.g.*, the lung tissues) from the sacrificed rats can be examined for histological changes.

**[00461]** The antibodies, compositions, or combination therapies of the invention can be tested for their ability to decrease the time course of viral infection. The antibodies, compositions, or combination therapies of the invention can also be tested for their ability to increase the survival period of humans suffering from a viral infection by at least 25%,

preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, antibodies, compositions, or combination therapies of the invention can be tested for their ability reduce the hospitalization period of humans suffering from viral infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the antibodies, compositions, or combination therapies of the invention *in vivo*.

**[00462]** Animal models for bacterial infections can also be used to assess the efficacy of an antibody, a composition, or a combination therapy of the invention. Animal models for bacterial infections such as *H. pylori*-infection, genital mycoplasmosis, primary sclerosing cholangitis, cholera, chronic lung infection with *Pseudomonas aeruginosa*, Legionnaires' disease, gastroduodenal ulcer disease, bacterial meningitis, gastric *Helicobacter* infection, pneumococcal otitis media, experimental allergic neuritis, leprous neuropathy, mycobacterial infection, endocarditis, *Aeromonas*-associated enteritis, *Bacteroides fragilis* infection, syphilis, streptococcal endocarditis, acute hematogenous osteomyelitis, human scrub typhus, toxic shock syndrome, anaerobic infections, *Escherichia coli* infections, and *Mycoplasma pneumoniae* infections have been developed (see, *e.g.*, Sugiyama *et al.*, 2002, J Gastroenterol. 37 Suppl 13:6-9; Brown *et al.*, 2001, Am J Reprod Immunol. 46(3):232-41; Vierling, 2001, Best Pract Res Clin Gastroenterol. 15(4):591-610; Klose, 2000, Trends Microbiol. 8(4):189-91; Stotland *et al.*, 2000, Pediatr Pulmonol. 30(5):413-24; Brieland *et al.*, 2000, Immunopharmacology 48(3):249-52; Lee, 2000, Baillieres Best Pract Res Clin Gastroenterol. 14(1):75-96; Koedel & Pfister, 1999, Infect Dis Clin North Am. 13(3):549-77; Nedrud, 1999, FEMS Immunol Med Microbiol. 24(2):243-50; Prellner *et al.*, 1999, Microb Drug Resist. 5(1):73-82; Vriesendorp, 1997, J Infect Dis. 176 Suppl 2:S164-8; Shetty & Antia, 1996, Indian J Lepr. 68(1):95-104; Balasubramanian *et al.*, 1994, Immunobiology 191(4-5):395-401; Carbon *et al.*, 1994, Int J Biomed Comput. 36(1-2):59-67; Haberberger *et al.*, 1991, Experientia. 47(5):426-9; Onderdonk *et al.*, 1990, Rev Infect Dis. 12 Suppl 2:S169-77; Wicher & Wicher, 1989, Crit Rev Microbiol. 16(3):181-234; Scheld, 1987, J Antimicrob Chemother. 20 Suppl A:71-85; Emslie & Nade, 1986, Rev Infect Dis. 8(6):841-9; Ridgway *et al.*, 1986, Lab Anim Sci. 36(5):481-5; Quimby & Nguyen, 1985, Crit Rev Microbiol. 12(1):1-44; Onderdonk *et al.*, 1979, Rev Infect Dis. 1(2):291-301; Smith, 1976, Ciba Found Symp. (42):45-72, and Taylor-Robinson, 1976, Infection. 4(1 Suppl):4-8).

**[00463]** The antibodies, compositions, or combination therapies of the invention can be tested for their ability to decrease the time course of bacterial infection, preferably bacterial respiratory infection by at least 25%, preferably at least 50%, at least 60%, at least

75%, at least 85%, at least 95%, or at least 99%. The antibodies, compositions, or combination therapies of the invention can also be tested for their ability to increase the survival period of humans suffering from a bacterial infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, the antibodies, compositions, or combination therapies administered according to the methods of the invention can be tested for their ability reduce the hospitalization period of humans suffering from bacterial infection, preferably a bacterial respiratory infection, by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the Antibodies of the invention, compositions, or combination therapies of the invention *in vivo*.

**[00464]** The efficacy of the antibodies, compositions, or combination therapies of the invention for the prevention, management, treatment, or amelioration of a fungal infection can be assessed in animal models for such infections. Animal models for fungal infections such as *Candida* infections, zygomycosis, *Candida* mastitis, progressive disseminated trichosporonosis with latent trichosporonemia, disseminated candidiasis, pulmonary paracoccidioidomycosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, cryptococcal meningitis, coccidioidal meningoencephalitis and cerebrospinal vasculitis, *Aspergillus niger* infection, *Fusarium* keratitis, paranasal sinus mycoses, *Aspergillus fumigatus* endocarditis, tibial dyschondroplasia, *Candida glabrata* vaginitis, oropharyngeal candidiasis, X-linked chronic granulomatous disease, tinea pedis, cutaneous candidiasis, mycotic placentitis, disseminated trichosporonosis, allergic bronchopulmonary aspergillosis, mycotic keratitis, *Cryptococcus neoformans* infection, fungal peritonitis, *Curvularia geniculata* infection, staphylococcal endophthalmitis, sporotrichosis, and dermatophytosis have been developed (see, *e.g.*, Arendrup *et al.*, 2002, *Infection* 30(5):286-91; Kamei, 2001, *Mycopathologia* 152(1):5-13; Guhad *et al.*, 2000, *FEMS Microbiol Lett.* 192(1):27-31; Yamagata *et al.*, 2000, *J Clin Microbiol.* 38(9):32606; Andrutis *et al.*, 2000, *J Clin Microbiol.* 38(6):2317-23; Cock *et al.*, 2000, *Rev Inst Med Trop Sao Paulo* 42(2):59-66; Shibuya *et al.*, 1999, *Microb Pathog.* 27(3):123-31; Beers *et al.*, 1999, *J Lab Clin Med.* 133(5):423-33; Najvar *et al.*, 1999, *Antimicrob Agents Chemother.* 43(2):413-4; Williams *et al.*, 1988, *J Infect Dis.* 178(4):1217-21; Yoshida, 1988, *Kansenshogaku Zasshi.* 1998 Jun;72(6):621-30; Alexandrakis *et al.*, 1998, *Br J Ophthalmol.* 82(3):306-11; Chakrabarti *et al.*, 1997, *J Med Vet Mycol.* 35(4):295-7; Martin *et al.*, 1997, *Antimicrob Agents Chemother.* 41(1):13-6; Chu *et al.*, 1996, *Avian Dis.* 40(3):715-9; Fidel *et al.*, 1996, *J Infect Dis.* 173(2):425-31; Cole *et al.*, 1995, *FEMS Microbiol Lett.* 15;126(2):177-80; Pollock *et al.*, 1995, *Nat Genet.* 9(2):202-9; Uchida *et al.*, 1994, *Jpn J Antibiot.*

47(10):1407-12; : Maebashi *et al.*, 1994, J Med Vet Mycol. 32(5):349-59; Jensen & Schonheyder, 1993, J Exp Anim Sci. 35(4):155-60; Gokaslan & Anaissie, 1992, Infect Immun. 60(8):3339-44; Kurup *et al.*, 1992, J Immunol. 148(12):3783-8; Singh *et al.*, 1990, Mycopathologia. 112(3):127-37; Salkowski & Balish, 1990, Infect Immun. 58(10):3300-6; Ahmad *et al.*, 1986, Am J Kidney Dis. 7(2):153-6; Altire-Werber E, Edberg SC, 1985, Mycopathologia. 89(2):69-73; Kane *et al.*, 1981, Antimicrob Agents Chemother. 20(5):595-9; Barbee *et al.*, 1977, Am J Pathol. 86(1):281-4; and Maestrone *et al.*, 1973, Am J Vet Res. 34(6):833-6). Animal models for fungal respiratory infections such as *Candida albicans*, *Aspergillus fumigatus*, invasive pulmonary aspergillosis, *Pneumocystis carinii*, pulmonary cryptococcosis, *Pseudomonas aeruginosa*, *Cunninghamella bertholletia* (see, e.g., Aratani *et al.*, 2002 Med Mycol 40(6):557-563; Bozza *et al.*, 2002 Microbes Infect 4(13): 1281-1290; Kurup *et al.*, 2002 Int Arch Allergy Immunol 129(2):129-137; Hori *et al.*, 2002 Eur J Immuno 32(5): 1282-1291; Rivera *et al.*, 2002 J Immuno 168(7): 3419-3427; Vassallo *et al.*, 2001, Am J Respir Cell Mol Biol 25(2): 203-211; Wilder *et al.*, 2002 Am J Respir Cell Mol Biol 26(3): 304-314; Yonezawa *et al.*, 2000 J Infect Chemother 6(3): 155-161; Cacciapuoti *et al.*, 2000 Antimicrob Agents Chemother 44(8): 2017-2022; and Honda *et al.*, 1998 Mycopathologia 144(3):141-146).

**[00465]** The antibodies, compositions, or combination therapies of the invention can be tested for their ability to decrease the time course of fungal respiratory infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. The antibodies, compositions, or combination therapies of the invention can also be tested for their ability to increase the survival period of humans suffering from a fungal respiratory infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, antibodies, compositions, or combination therapies administered according to the methods of the invention can be tested for their ability reduce the hospitalization period of humans suffering from fungal respiratory infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the antibodies, compositions, or combination therapies of the invention *in vivo*.

**[00466]** Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of an antibody, a composition, a combination therapy disclosed herein for prevention, treatment, management, and/or amelioration of disorder or disease characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disorder or disease characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder,

a proliferative disorder, or an infection (preferably, a respiratory infection) or one or more symptoms thereof.

#### **5.6.4 Toxicity Assays**

**[00467]** The toxicity and/or efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Therapies that exhibit large therapeutic indices are preferred. While therapies that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

**[00468]** The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any therapy used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

**[00469]** Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of an antibody, a composition, a combination therapy disclosed herein for a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disease or disorder associated with or characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection) or one or more symptoms thereof.



## **5.7 DIAGNOSTIC USES OF ANTIBODIES**

**[00470]** Antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to an IL-9 polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more symptoms thereof. The invention provides for the detection of aberrant expression of IL-9 comprising: (a) assaying the expression of IL-9 in a biological sample from an individual using one or more antibodies of the invention that immunospecifically binds to an IL-9 polypeptide; and (b) comparing the level of IL-9 with a standard level of IL-9, *e.g.*, in normal biological samples, whereby an increase or decrease in the assayed level of IL-9 compared to the standard level of IL-9 is indicative of a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more symptoms thereof. In specific embodiments, aberrant expression level of IL-9 is indicative of an autoimmune disorder or a disease or condition associated therewith. In another specific embodiment, an aberrant expression level of IL-9 is indicative of an inflammatory disorder or a disease or condition associated therewith, such as asthma. In preferred embodiments, an aberrant expression level of IL-9 is indicative of a respiratory infection, such as, but not limited to RSV, PVI, or hMPV.

**[00471]** In preferred embodiments, the labeled antibodies of the invention that immunospecifically bind to IL-9 are used for diagnostic purposes to detect, diagnose, prognose, or monitor a respiratory infection, preferably RSV infection, PIV infection, or hMPV. The invention provides methods for the detection of a respiratory infection, comprising: (a) assaying the expression of IL-9 in cells or a tissue sample of a subject using one or more antibodies that immunospecifically bind to IL-9; and (b) comparing the level of IL-9 with a control level, *e.g.*, levels in normal tissue samples not infected, whereby an increase in the assayed level of IL-9 compared to the control level of IL-9 is indicative of a respiratory infection.

**[00472]** Antibodies of the invention can be used to assay IL-9 levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, see Jalkanen *et al.*, 1985, J. Cell. Biol. 101:976-985; and

Jalkanen *et al.*, 1987, J. Cell . Biol. 105:3087-3096). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{121}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

**[00473]** One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of IL-9 in an animal, preferably a mammal, and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically binds to an IL-9 polypeptide; b) waiting for a time interval following the administering for permitting the labeled antibody to preferentially concentrate at sites in the subject where IL-9 is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody or fragment thereof above the background level and above or below the level observed in a person without the disease or disorder indicates that the subject has a particular disease or disorder associated with aberrant expression of IL-9. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system. Aberrant expression of IL-9 can occur particularly in lymphoid and myeloid cell types. A more definitive diagnosis of respiratory infection may allow health professionals to employ preventive measures or aggressive treatment earlier and thereby prevent the development or further progression of the infection.

**[00474]** It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99}\text{Tc}$ . The labeled antibody will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds, Masson Publishing Inc. (1982). Depending on several variables, including the

type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours, 6 to 24 hours, or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

**[00475]** In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disorder, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

**[00476]** Presence of the labeled IL-9 antibody can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

**[00477]** In a specific embodiment, the IL-9 antibody is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the IL-9 antibody is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the IL-9 antibody is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the IL-9 antibody is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

**[00478]** The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may be utilized for immunophenotyping of cell lines and biological samples by their IL-9 expression or IL-9 receptor expression. Various techniques can be utilized using the antibodies, fragments, or variants of the invention to screen for cellular populations (that express IL-9 and/or IL-9 receptor, particularly immune cells, *i.e.*, T and B lymphocytes, mast cells, eosinophils, macrophages, neutrophils and epithelial cells or IL-9 receptor, and include magnetic separation using antibody-coated magnetic beads, “panning” with antibody attached to a solid matrix (*i.e.*, plate), and flow cytometry (see, *e.g.*, U.S. Patent 5,985,660; and Morrison *et al.*, Cell, 96:737-49 (1999)).

**[00479]** These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (*i.e.*, minimal residual disease

(MRD) in acute leukemic patients) and “non-self” cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

## **5.8     KITS**

[00480]        The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In another embodiment, a kit comprises an antibody fragment of the invention that immunospecifically binds to an IL-9 polypeptide. In a specific embodiment, the kits of the present invention contain a substantially isolated IL-9 polypeptide as a control. Preferably, the kits of the present invention further comprise a control antibody which does not react with an IL-9 polypeptide. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to an IL-9 polypeptide (*e.g.*, the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized IL-9 polypeptide. The IL-9 polypeptide provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which an IL-9 polypeptide is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the IL-9 polypeptide can be detected by binding of the said reporter-labeled antibody.

[00481]        In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing an IL-9 polypeptide. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with an IL-9 polypeptide, and means for detecting the binding of the IL-9 polypeptide to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

## **5.9     ARTICLES OF MANUFACTURE**

[00482]        The present invention also encompasses a finished packaged and labeled pharmaceutical product. This article of manufacture includes the appropriate unit dosage

form in an appropriate vessel or container such as a glass vial or other container that is hermetically sealed. The pharmaceutical product may be formulated in single dose vials as a sterile liquid that contains 10 mM histidine buffer at pH 6.0 and 150 mM sodium chloride. Each 1.0 mL of solution may contain 100 mg of protein, 1.6 mg of histidine and 8.9 mg of sodium chloride in water for injection. During the manufacturing process the pH of the formulation buffer is adjusted to 6.0 using hydrochloric acid. In the case of dosage forms suitable for parenteral administration the active ingredient, *e.g.*, an antibody of the invention that immunospecifically binds to an IL-9 polypeptide, is sterile and suitable for administration as a particulate free solution. In other words, the invention encompasses both parenteral solutions and lyophilized powders, each being sterile, and the latter being suitable for reconstitution prior to injection. Alternatively, the unit dosage form may be a solid suitable for oral, transdermal, intranasal, or topical delivery.

[00483] In a preferred embodiment, the unit dosage form is suitable for intravenous, intramuscular, intranasal, oral, topical or subcutaneous delivery. Thus, the invention encompasses solutions, preferably sterile, suitable for each delivery route.

[00484] As with any pharmaceutical product, the packaging material and container are designed to protect the stability of the product during storage and shipment. Further, the products of the invention include instructions for use or other informational material that advise the physician, technician or patient on how to appropriately prevent or treat the disease or disorder in question. In other words, the article of manufacture includes instruction means indicating or suggesting a dosing regimen including, but not limited to, actual doses, monitoring procedures, total lymphocyte, mast cell counts, T cell counts, IgE production, and other monitoring information.

[00485] Specifically, the invention provides an article of manufacture comprising packaging material, such as a box, bottle, tube, vial, container, sprayer, insufflator, intravenous (i.v.) bag, envelope and the like; and at least one unit dosage form of a pharmaceutical agent contained within said packaging material, wherein said pharmaceutical agent comprises an antibody that immunospecifically binds to IL-9 and wherein said packaging material includes instruction means which indicate that said antibody can be used to prevent, manage, treat, and/or ameliorate one or more symptoms associated with a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more

symptoms thereof by administering specific doses and using specific dosing regimens as described herein.

**[00486]** The invention also provides an article of manufacture comprising packaging material, such as a box, bottle, tube, vial, container, sprayer, insufflator, intravenous (i.v.) bag, envelope and the like; and at least one unit dosage form of each pharmaceutical agent contained within said packaging material, wherein one pharmaceutical agent comprises an antibody that immunospecifically binds to an IL-9 polypeptide and the other pharmaceutical agent comprises a second, different antibody that immunospecifically binds to an IL-9 polypeptide, and wherein said packaging material includes instruction means which indicate that said agents can be used to treat, prevent and/or ameliorate a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more symptoms thereof by administering specific doses and using specific dosing regimens as described herein.

**[00487]** The invention also provides an article of manufacture comprising packaging material, such as a box, bottle, tube, vial, container, sprayer, insufflator, intravenous (i.v.) bag, envelope and the like; and at least one unit dosage form of each pharmaceutical agent contained within said packaging material, wherein one pharmaceutical agent comprises an antibody that immunospecifically binds to an IL-9 polypeptide and the other pharmaceutical agent comprises a prophylactic or therapeutic agent other than an antibody that immunospecifically binds to an IL-9 polypeptide, and wherein said packaging material includes instruction means which indicate that said agents can be used to treat, prevent and/or ameliorate one or more symptoms associated with a disorder associated with aberrant expression and/or activity of an IL-9 polypeptide, a disorder associated with aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an autoimmune disorder, an inflammatory disorder, a proliferative disorder, an infection (preferably, a respiratory infection), or one or more symptoms thereof by administering specific doses and using specific dosing regimens as described herein.

**[00488]** The present invention provides that the adverse effects that may be reduced or avoided by the methods of the invention are indicated in informational material enclosed in an article of manufacture for use in preventing, treating and/or ameliorating one or more symptoms associated with an autoimmune disorder, an inflammatory disorder or an infection. Adverse effects that may be reduced or avoided by the methods of the invention include, but are not limited to, vital sign abnormalities (fever, tachycardia, bradycardia,

hypertension, hypotension), hematological events (anemia, lymphopenia, leukopenia, thrombocytopenia), headache, chills, dizziness, nausea, asthenia, back pain, chest pain (chest pressure), diarrhea, myalgia, pain, pruritus, psoriasis, rhinitis, sweating, injection site reaction, and vasodilatation. Since antibodies of the invention that immunospecifically bind to an IL-9 polypeptide may be immunosuppressive, prolonged immunosuppression may increase the risk of infection, including opportunistic infections. Prolonged and sustained immunosuppression may also result in an increased risk of developing certain types of cancer.

**[00489]** Further, the information material enclosed in an article of manufacture for use in preventing, treating, managing, and/or ameliorating disorder characterized by aberrant expression and/or activity of an IL-9 polypeptide, a disorder characterized by aberrant expression and/or activity of an IL-9R or one or more subunits thereof, an inflammatory disorder, an autoimmune disorder, a proliferative disorder, or an infection (preferably, a respiratory infection) or one or more symptoms thereof can indicate that foreign proteins may also result in allergic reactions, including anaphylaxis, or cytosine release syndrome. The information material should indicate that allergic reactions may exhibit only as mild pruritic rashes or they may be severe such as erythroderma, Stevens-Johnson syndrome, vasculitis, or anaphylaxis. The information material should also indicate that anaphylactic reactions (anaphylaxis) are serious and occasionally fatal hypersensitivity reactions. Allergic reactions including anaphylaxis may occur when any foreign protein is injected into the body. They may range from mild manifestations such as urticaria or rash to lethal systemic reactions. Anaphylactic reactions occur soon after exposure, usually within 10 minutes. Patients may experience paresthesia, hypotension, laryngeal edema, mental status changes, facial or pharyngeal angioedema, airway obstruction, bronchospasm, urticaria and pruritus, serum sickness, arthritis, allergic nephritis, glomerulonephritis, temporal arthritis, or eosinophilia.

## **5.10 METHODS OF PRODUCING ANTIBODIES**

**[00490]** Antibodies that immunospecifically bind to an antigen can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

**[00491]** Polyclonal antibodies that immunospecifically bind to an antigen can be produced by various procedures well-known in the art. For example, a human antigen can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the human

antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

**[00492]** Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

**[00493]** Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with an IL-9 polypeptide and once an immune response is detected, *e.g.*, antibodies specific for IL-9 are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. Additionally, a RIMMS (repetitive immunization multiple sites) technique can be used to immunize an animal (Kilpatrick *et al.*, 1997 *Hybridoma* 16:381-9, incorporated by reference in its entirety). The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

**[00494]** Accordingly, the present invention provides methods of generating antibodies by culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an IL-9 polypeptide with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to an IL-9 polypeptide.



**[00495]** Antibody fragments which recognize specific IL-9 epitopes may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

**[00496]** In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman *et al.*, 1995, J. Immunol. Methods 182:41-50; Ames *et al.*, 1995, J. Immunol. Methods 184:177-186; Kettleborough *et al.*, 1994, Eur. J. Immunol. 24:952-958; Persic *et al.*, 1997, Gene 187:9-18; Burton *et al.*, 1994, Advances in Immunology 57:191-280; PCT Application No. PCT/GB91/O1 134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

**[00497]** As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax *et al.*, 1992, BioTechniques 12(6):864-869;

Sawai *et al.*, 1995, AJRI 34:26-34; and Better *et al.*, 1988, Science 240:1041-1043 (said references incorporated by reference in their entireties).

**[00498]** To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1 $\alpha$  promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

**[00499]** For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

**[00500]** Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J<sub>H</sub> region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to

produce chimeric mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, PCT publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

**[00501]** A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, 1985, *Science* 229:1202; Oi *et al.*, 1986, *BioTechniques* 4:214; Gillies *et al.*, 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, which are incorporated herein by reference in their entirety.

**[00502]** A humanized antibody is an antibody or its variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab').sub.2, Fabc, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and

CH4 regions of the heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG.sub.1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG.sub.2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibody can be produced using variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; and Roguska *et al.*, 1994, *PNAS* 91:969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, WO 9317105, Tan *et al.*, *J. Immunol.* 169:1119-25 (2002), Caldas *et al.*, *Protein Eng.* 13(5):353 - 60 (2000), Morea *et al.*, *Methods* 20(3):267-79 (2000), Baca *et al.*, *J. Biol. Chem.* 272(16):10678-84 (1997), Roguska *et al.*, *Protein Eng.* 9(10):895-904 (1996), Couto *et al.*, *Cancer Res.* 55 (23 Supp):5973s - 5977s (1995), Couto *et al.*, *Cancer Res.* 55(8):1717-22 (1995), Sandhu JS, *Gene* 150(2):409-10 (1994), and Pedersen *et al.*, *J. Mol. Biol.* 235(3):959-73 (1994). Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; and Riechmann *et al.*, 1988, *Nature* 332:323, which are incorporated herein by reference in their entireties.)

**[00503]** Single domain antibodies, for example, antibodies lacking the light chains, can be produced by methods well-known in the art. See Riechmann *et al.*, 1999, J. Immuno. 231:25-38; Nuttall *et al.*, 2000, Curr. Pharm. Biotechnol. 1(3):253-263; Muylderman, 2001, J. Biotechnol. 74(4):277302; U.S. Patent No. 6,005,079; and International Publication Nos. WO 94/04678, WO 94/25591, and WO 01/44301, each of which is incorporated herein by reference in its entirety.

**[00504]** Further, the antibodies that immunospecifically bind to an antigen (*e.g.*, IL-9 polypeptide) can, in turn, be utilized to generate anti-idiotypic antibodies that “mimic” an antigen using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan & Bona, 1989, FASEB J. 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438).

#### **5.10.1 Polynucleotide Sequences Encoding Antibodies**

**[00505]** The invention provides polynucleotides comprising a nucleotide sequence encoding an antibody or fragment thereof that immunospecifically binds to an antigen (*e.g.*, IL-9 polypeptide). The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode an antibody of the invention.

**[00506]** The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. Since the amino acid sequences of 4D4, 4D4 H2-1 D11, 4D4com-XF-9, 4D4com-2F9, 7F3, 71A10, 22D3, 7F3com-3H5, and 7F3com-3D4 are known, nucleotide sequences encoding these antibodies can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, fragments, or variants thereof, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

**[00507]** Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the

antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

**[00508]** Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

**[00509]** In a specific embodiment, one or more of the CDRs is inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia *et al.*, 1998, J. Mol. Biol. 278: 457-479 for a listing of human framework regions). Preferably, the polynucleotide sequence generated by the combination of the framework regions and CDRs encodes an antibody that immunospecifically binds to a particular antigen (*e.g.*, an IL-9 polypeptide). Preferably, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

#### **5.10.2 Recombinant Expression of Antibodies**

**[00510]** Recombinant expression of an antibody of the invention (*e.g.*, a heavy or light chain of an antibody of the invention or a fragment thereof or a single chain antibody of the invention) that immunospecifically binds to an IL-9 polypeptide requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a

polynucleotide encoding an antibody molecule, heavy or light chain of an antibody, or fragment thereof (preferably, but not necessarily, containing the heavy or light chain variable domain) of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well-known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody or a fragment thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, International Publication No. WO 86/05807; International Publication No. WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

**[00511]** The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention or fragments thereof, or a heavy or light chain thereof, or fragment thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

**[00512]** A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention (see, *e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences;

yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, 1986, Gene 45:101; and Cockett *et al.*, 1990, Bio/Technology 8:2). In a specific embodiment, the expression of nucleotide sequences encoding antibodies of the invention, derivative, analog, or fragment thereof which immunospecifically bind to an IL-9 polypeptide or fragments thereof is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

**[00513]** In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such an antibody is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, EMBO 12:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor



Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

**[00514]** In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

**[00515]** In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner *et al.*, 1987, Methods in Enzymol. 153:51-544).

**[00516]** In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D,

NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O and HsS78Bst cells.

**[00517]** For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

**[00518]** A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, 1977, Cell 11:223), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy *et al.*, 1980, Cell 22:8-17) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, 1980, Natl. Acad. Sci. USA 77:357; O'Hare *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191-217; May, 1993, TIB TECH 11(5):155-2 15); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-

Garapin *et al.*, 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

**[00519]** The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, 1983, Mol. Cell. Biol. 3:257).

**[00520]** The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; and Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2 197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

**[00521]** Once an antibody molecule of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

### **5.11 IL-9 POLYPEPTIDES**

**[00522]** An IL-9 polypeptide may be IL-9, an analog, derivative or a fragment thereof, or a fusion protein comprising IL-9, an analog, derivative or a fragment thereof. The IL-9 polypeptide may be from any species. The nucleotide and/or amino acid sequences of IL-9 polypeptides can be found in the literature or public databases, or the nucleotide and/or amino acid sequences can be determined using cloning and sequencing

techniques known to one of skill in the art. For example, the nucleotide sequence of human IL-9 can be found in the GenBank database (see, *e.g.*, Accession No. NM\_000590; FIG. 12). The amino acid sequence of human IL-9 can be found in the GenBank database (see, *e.g.*, Accession Nos. A60480 and AAC17735; FIG. 13) and in U.S. Provisional Application No. 60/371,683 (the amino acid sequence of human IL-9 on page 15 is specifically incorporated herein by reference). In a preferred embodiment, an IL-9 polypeptide is human IL-9, an analog, derivative or a fragment thereof.

**[00523]** An IL-9 polypeptide may be a “free-standing” fragment of IL-9, or a fragment of IL-9 within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of IL-9 fragments that may be bound by antibodies of the present invention, include, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 15, 16-30, 31-46, 47-55, 56-72, 73-104, 105-126 the of the amino acid sequence corresponding to human IL-9. Moreover, an IL-9 polypeptide that may be bound by antibodies of the present invention can be at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120 or at least 125 amino acids in length. In this context, “about” means the particularly recited ranges and ranges larger or smaller by several, a few, 5, 4, 3, 2 or 1 amino acid residues at either or both the amino- and carboxy-termini.

**[00524]** An IL-9 polypeptide may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CHI, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, *e.g.*, U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4 polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, *e.g.*, EP 394,827; Traunecker *et al.*, Nature, 331:84-86 (1998). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (*e.g.*, insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, *e.g.*, International Publication Nos. WO 96/22024 and WO 99/048 13). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more

efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, *e.g.*, Fountoulakis *et al.*, J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (*e.g.*, the hemagglutinin (“HA”) tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht *et al.*, allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is transitionally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine tagged proteins can be selectively eluted with imidazole-containing buffers.

**[00525]** In a specific embodiment, an IL-9 polypeptides is fused with a heterologous antigen (*e.g.*, polypeptide, carbohydrate, phospholipid, or nucleic acid). In specific embodiments, the heterologous antigen is an immunogen.

**[00526]** In another embodiment, an IL-9 polypeptide is a derivative of IL-9 or the epitope-bearing fragments thereof. Such derivatives can be generated by random mutagenesis of a polynucleotide encoding IL-9, by error-prone PCR, random nucleotide insertion or other methods prior to recombination. Alternatively, site-directed mutagenesis techniques can be used to produce derivatives. Amino acids in the IL-9 polypeptides that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for functional activity, such ligand binding and the ability to stimulate lymphocyte (*e.g.*, B cell) as, for example, proliferation, differentiation, and/or activation. In one embodiment of the invention, an IL-9 polypeptide comprises an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, even more preferably, not more than 40 conservative amino acid substitutions, still more preferably, not more than 30 conservative amino acid substitutions, and still even more preferably, not more than 20 conservative amino acid substitutions relative to the native IL-9 amino acid sequence (*e.g.*, the native human IL-9 amino acid sequence). In another embodiment of the invention, an IL-9 polypeptide comprises an amino acid sequence which contains at least one conservative amino acid substitution; but not more

than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions relative to the native IL-9 amino acid sequence (*e.g.*, the native human IL-9 amino acid sequence). In yet another embodiment, an IL-9 polypeptide comprises an amino acid sequence which contains one or more conservative substitutions or a combination of non-conservative and conservative amino acid substitutions relative to the native IL-9 amino acid sequence.

**[00527]** To improve or alter the characteristics of IL-9 polypeptides, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or “muteins” including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, *e.g.*, enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. For instance, for many proteins, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron *et al.*, J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing. Accordingly, antibodies of the present invention may bind IL-9 polypeptide mutants or variants generated by protein engineering.

**[00528]** In another embodiment, an IL-9 polypeptide is at least 50%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identical to a native IL-9 amino acid sequence (*e.g.*, a native human IL-9 amino acid sequence).

## **5.12 METHODS OF PRODUCING POLYPEPTIDES**

Polypeptides, peptides, proteins, and fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a polypeptides, peptides, proteins, or fusion proteins can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992).

**[00529]** The nucleotide sequences encoding a polypeptide, peptide, protein, or fusion protein may be obtained from any information available to those of skill in the art (*i.e.*, from Genbank, the literature, or by routine cloning). The nucleotide sequence coding for a

polypeptide, peptide, protein, and fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

**[00530]** The expression of a polypeptide, peptide, protein, or fusion protein may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding fusion protein include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, *Nature* 296:39-42), the tetracycline (Tet) promoter (Gossen *et al.*, 1995, *Proc. Nat. Acad. Sci. USA* 89:5547-5551); prokaryotic expression vectors such as the  $\beta$ -lactamase promoter (Villa-Kamaroff, *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.* 75:3727-3731), or the *tac* promoter (DeBoer, *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.* 80:21-25; see also "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.*, *Nature* 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.*, 1981, *Nucl. Acids Res.* 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, 1984, *Nature* 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-646; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-

658; Adames *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogam *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli *et al.*, 1999, Gen. Virol. 80:571-83); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi *et al.*, 1998, Biochem. Biophysic. Res. Com. 253:818-823); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes *et al.*, 1999, Braz J Med Biol Res 32(5):619-631; Morelli *et al.*, 1999, Gen. Virol. 80:571-83) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378).

**[00531]** In a specific embodiment, the expression of a polypeptide, peptide, protein, or fusion protein is regulated by a constitutive promoter. In another embodiment, the expression of a polypeptide, peptide, protein, or a fusion protein is regulated by an inducible promoter. In another embodiment, the expression of a polypeptide, peptide, protein, or a fusion protein is regulated by a tissue-specific promoter.

**[00532]** In a specific embodiment, a vector is used that comprises a promoter operably linked to a polypeptide, peptide, protein, or a fusion protein-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene).

**[00533]** In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the polypeptide or fusion protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected



hosts (*e.g.*, see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted fusion protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner *et al.*, 1987, Methods in Enzymol. 153:51-544).

**[00534]** Expression vectors containing inserts of a gene encoding a polypeptide, peptide, protein, or fusion protein can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of “marker” gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a polypeptide, peptide, protein, or a fusion protein in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding the polypeptide, peptide, protein, or the fusion protein, respectively. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain “marker” gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a nucleotide sequence encoding a polypeptide, peptide, protein, or fusion protein in the vector. For example, if the nucleotide sequence encoding the fusion protein is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the fusion protein insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (*e.g.*, fusion protein) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the fusion protein in *in vitro* assay systems, *e.g.*, binding to an antibody.

**[00535]** In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered fusion protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For

example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, NS0, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto *et al.*, 1984, J. Natl. Cancer Inst. 73: 51-57), SK-N-SH human neuroblastoma (Biochim. Biophys. Acta, 1982, 704: 450-460), Daoy human cerebellar medulloblastoma (He *et al.*, 1992, Cancer Res. 52: 1144-1148) DBTRG-05MG glioblastoma cells (Kruse *et al.*, 1992, In Vitro Cell. Dev. Biol. 28A: 609-614), IMR-32 human neuroblastoma (Cancer Res., 1970, 30: 2110-2118), 1321N1 human astrocytoma (Proc. Natl Acad. Sci. USA, 1977, 74: 4816), MOG-G-CCM human astrocytoma (Br. J. Cancer, 1984, 49: 269), U87MG human glioblastoma-astrocytoma (Acta Pathol. Microbiol. Scand., 1968, 74: 465-486), A172 human glioblastoma (Olopade *et al.*, 1992, Cancer Res. 52: 2523-2529), C6 rat glioma cells (Benda *et al.*, 1968, Science 161: 370-371), Neuro-2a mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1970, 65: 129-136), NB41A3 mouse neuroblastoma (Proc. Natl. Acad. Sci. USA, 1962, 48: 1184-1190), SCP sheep choroid plexus (Bolin *et al.*, 1994, J. Virol. Methods 48: 211-221), G355-5, PG-4 Cat normal astrocyte (Haapala *et al.*, 1985, J. Virol. 53: 827-833), Mpf ferret brain (Trowbridge *et al.*, 1982, In Vitro 18: 952-960), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89: 6467-6471) such as, for example, CRL7030 and Hs578Bst. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

**[00536]** For long-term, high-yield production of recombinant polypeptide, peptide, protein, or fusion protein, stable expression is preferred. For example, cell lines which stably express a polypeptide, peptide, protein, or a fusion protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express a polypeptide, peptide, protein,

or a fusion protein that immunospecifically binds to an IL-9 polypeptide. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the activity of a polypeptide, peptide, protein, or fusion protein that immunospecifically binds to an IL-9 polypeptide.

[00537] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, Cell 22:817) genes can be employed in tk-, hgp<sup>rt</sup>- or ap<sup>rt</sup>- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, *et al.*, 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, 1981, J. Mol. Biol. 150:1); and hyg<sup>ro</sup>, which confers resistance to hygromycin (Santerre, *et al.*, 1984, Gene 30:147) genes.

[00538] Once a polypeptide, peptide, protein, or a fusion protein of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of a protein, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

## **6. EXAMPLES**

[00539] Certain embodiments of the invention are illustrated by the following non-limiting examples.

### **6.1 EXAMPLE 1: PREPARATION OF ANTI-IL-9 ANTIBODY 7F3COM-2H2**

[00540] The anti-IL-9 humanized monoclonal antibody 7F3com-2H2 was prepared using the pMI347 vector. The pMI347 vector coding for the expression of 7F3com-2H2 consists of the following four independent genetic elements: a glutamine synthetase selectable marker expression cassette, the 2H2 kappa light chain cDNA expression cassette, the 2H2  $\gamma$ 1 heavy chain mini-gene expression cassette, and a bacterial origin of replication and antibiotic resistance gene.

[00541] The first element, the glutamine synthetase selectable marker expression cassette consists of hamster glutamine synthetase (GS) cDNA under the control of simian virus 40 (SV40) early enhancer and promoter and SV40 early splicing and polyadenylation region for efficient mRNA cleavage and addition of a polyadneylate tail. This element is required for integration, amplification, and stable maintenance of the plasmid in the host cell genome.

[00542] Each of the 2H2 heavy and light chain expression cassettes consist of the human cytomegalovirus major immediate early (hCMVie) enhancer, promoter, and 5'-untranslaterd region directing the high level transcription of 2H2 light cahin cDNA, and athe SV40 early polyadenylation region for efficient polyadenylation. This combination of a strong enhancer/promoter and an efficient polyadenylation region assures high levels of stable 2H2 light chain mRNA in the cells. The light and heavy chain expression cassettes are separated by a murine immunoglobulin mu transcription termination region to prevent transcriptional interference of the downstream genes.

[00543] The final element of the pMI347 vector is the bacterial origin of replication and antibiotic resistance gene (*e.g.*, beta-lactamase gene) allow for the propagation and selection of the vector *E. coli*.

[00544] The pMI347 vector encoding the anti-IL-9 monoclonal antibody 7F3com-2H2 has been deposited in the form of *E. coli* (DH5- $\alpha$ ) with the American Type Culture Collection (ATCC), located in Manassas, Virginia, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure ("Budapest Treaty"), on April 9, 2004, and assigned accession number PTA-5913.

#### **Transient Mammalian Cell Expression of 7F3com-2H2**

[00545] The expression vector pMI347 was introduced to 293-H cells vial lipid mediated transfection for transient expression. After 72 hours, the cell culture supernatant was harvested and fresh growth medium was added to each plate. The process was repeated after 96 hours. After the third harvest, the cell culture supernatants were pooled and assayed for human IgG content.

#### **Stable Mammalian Cell Expression of 7F3com-2H2**

[00546] The expression vector pMI347 was linearized with the Sal I restriction enzyme and introduced into NS0 cells by electroporation. Cells were selected for integration of the plasmid into the host cell genome in glutamine-free medium. Colonies

surviving in the glutamine-free environment were screened for immunoglobulin expression and the highest expressing colonies were expanded. Then, these primary transfectants were cloned by limiting dilution in glutamine-free medium to isolate high producing sub-populations. The clones were screened for specific productivity and growth characteristics. Those clones that exhibited the most favorable combinations of high productivity and rapid growth were selected for evaluation as production cell lines.

### **Quantitation of 7F3com-2H2**

[00547] Cell culture supernatants were screened for the 7F3com-2H2 antibody using a sandwich ELISA. Assay plates coated with goat-anti-human IgG were washed and incubated with cell culture supernatant and human IgG standards. After incubation, the plates were washed to remove non-bound IgG. Then the plates were reacted with horseradish peroxidase-labeled goat-anti-human IgG secondary antibody. After incubation, the plates were again washed. The chromogenic substrate 3, 3', 5, 5'-tetramethylbenzidine was added to each well and after five minutes, 0.1N sulfuric acid was added to terminate the substrate turnover. The substrate turnover was then measured at 450 nm using a microplate reader. The standard curve was plotted as a log-linear relationship and unknown samples were compared to the standard curve to estimate their human IgG content.

## **6.2 ANTIBODY PURIFICATION**

[00548] The following section describes a method for purifying antibodies to be used in the methods of the invention.

### **Buffer Components and Equipment**

[00549] Buffers, process solutions and cleaning solutions are prepared with water for injection (WFI). Buffers are tested for bioburden and endotoxin.

#### **Buffers and Process Solutions**

0.1 M citric acid  
10 mM sodium citrate, 80 mM NaCl, pH 4.6  
25 mM sodium phosphate, pH 6.5  
20 mM Tris-HCl, 40 mM NaCl, pH 7.5  
0.5 M sodium phosphate, pH 6.5  
5 mM sodium phosphate, 40 mM NaCl, pH 6.5  
50 mM Glycine-HCl, 30 mM NaCl, pH 2.5  
50 mM Glycine-HC, pH 2.35  
1.0 M Tris base

#### **Cleaning and Storage Solutions**

Water for Injection (WFI)  
1.0 N NaOH

0.1 N NaOH  
20% (v/v) ethanol  
0.5 N NaOH, 400 ppm sodium hypochlorite

**Formulation Buffers**

10 mM Histidine, 150 mM NaCl, pH 6.0  
4 M sodium chloride

**Equipment (Substitutions with equivalent performing materials are acceptable)**

300 kg scale  
Conductivity meter  
Stir plate  
pH meter  
Vessels: Appropriately sized Stedim™ bags, buffer tanks, PETG Bottles  
Watson Marlow 1700 peristaltic pump  
Wedgewood UV, pH, conductivity unit  
Amersham Pharmacia chromatography controller  
Packed POROS HS50 cation exchange gel  
Packed Pharmacia rProtein A affinity gel  
Packed POROS HQ anion exchange gel  
Sterile, depyrogenated silicone tubing  
Integritest Filter Integrity Tester II  
Sterile Asahi Planova 20 N membrane viral removal filter  
Millipore 0.2 micron Durapore filter  
Millipore Multimedia filter  
CUNO 60LP, 10/60 SP filter  
CUNO filter housing  
Class 100 hood

**Purification and Formulation of the Antibodies**

[00550] The purification process comprises three chromatography steps, a nanofiltration step, a low pH treatment step, and formulation. These steps are designed to remove host cell proteins, DNA and cell culture components such as BSA and transferrin. In addition, the process includes steps to control bioburden and endotoxin and to remove and inactivate viruses.

**Conditioned Medium (Steps 1 to 6)**

[00551] Conditioned medium from a single cell culture lot or pooled from multiple cell culture lots is purified as a single lot. The combination of multiple cell culture lots into one purification lot is performed in order to utilize downstream processing steps sized for a single lot size and to decrease the number of purification lots. For example, because the working volumes of 130 L and 250 L cell culture bioreactors are approximately 100 L and 200 L, respectively, these two cell culture lots could be pooled and run as one 300 L purification lot. Process product samples are analyzed for DNA using a PicoGreen or a quantitative PCR assay to detect DNA. Protein concentration is determined either by a

Protein A bindable HPLC assay or by UV absorbance at 280 nm. Product-containing process streams are monitored for endotoxin and bioburden. Column effluents are monitored for endotoxin. A description of each step is summarized below.

#### **Conditioned Medium Adjustment and Filtration (Step 7)**

[00552] The conditioned medium is adjusted to pH  $4.6 \pm 0.2$  with 0.1 M citric acid. The adjusted conditioned medium is then filtered using a CUNO filter in-line with a Millipore 0.2 micron Durapore filter.

#### **Cation Exchange Chromatography Step (Step 8)**

[00553] The adjusted and filtered conditioned medium is loaded onto a cation exchange column that has been equilibrated with 10 mM sodium phosphate, 80 mM sodium chloride, pH 4.6. The bound antibody is washed using the same buffer. The column is then washed with 25 mM sodium phosphate pH 6.5 to remove process impurities, especially BSA. The product is eluted using 20 mM Tris-HCl buffer, 40 mM NaCl, pH 7.5. Following elution of the product, the column is cleaned with 1.0 N NaOH and stored in 0.1 N NaOH at room temperature.

#### **rProtein A Chromatograph (Step 9)**

[00554] The cation exchange product is loaded directly onto a rProtein A column equilibrated with 20 mM Tris-HCl buffer, 40 mM NaCl, pH 7.5. Following loading, the column is washed with the equilibration buffer, and the product is eluted with 50 mM glycine, 30 mM NaCl, pH 3.2. The rProtein A product is neutralized to pH  $6.5 \pm 0.2$  with 1.0 M Tris base. This chromatography step removes additional process-related impurities. At the end of the step, the column is washed with equilibration buffer, cleaned with 0.1 N NaOH, washed with equilibration buffer and stored in 20% (v/v) ethanol at room temperature.

#### **Anion Exchange Chromatography (Step 10)**

[00555] This chromatographic step is the final step designed to remove any trace levels of process-related impurities. The column is equilibrated with 0.5 M sodium phosphate, pH 6.5 followed by equilibration with 5 mM sodium phosphate, 40 mM sodium chloride, pH 6.5. Under these conditions, the neutralized rProtein A product is loaded onto the equilibrated anion exchange column, and under these conditions, the product is recovered in the non-bound fraction and the process-related impurities are retained in the column. The column is cleaned with 1.0 N NaOH and stored in 0.1 N NaOH at room temperature.

### **Nanofiltration (Step 11)**

[00556] The anion exchange product is filtered through a sterile Planova™ 20 N membrane (pore size = 20 nm) that is prepared by flushing first with WFI and then with 5 mM sodium phosphate, 40 mM sodium chloride pH 6.5. After the product is filtered, the filter is chased with a small volume of 5 mM sodium phosphate, 40 mM sodium chloride, pH 6.5 to maximize product recovery. After filtration the nanofilter is integrity tested.

### **Low pH Treatment (Step 12)**

[00557] The pH of the nanofiltered product is adjusted to  $3.4 \pm 0.1$  with 50 mM glycine, pH 2.35 and held at this pH for  $30 \pm 10$  minutes. After low pH treatment, the product pH is adjusted to  $6.5 \pm 0.2$  with 1.0 M Tris base.

## **6.3 INTERACTION OF IL-9 ANTIBODIES WITH RHUIL9**

[00558] The interaction of soluble 7F3com-2H2 and MH9A3 (both in an IgG and Fab form) with immobilized rhuIL9 was monitored by surface plasmon resonance detection using a BIAcore 3000 instrument (Pharmacia Biosensor, Uppsala, Sweden). rhuIL9 was coupled to the dextran matrix of a CM5 sensor chip (Pharmacia Biosensor) using an Amine Coupling Kit at a surface density of between 100 and 200 RU. 7F3com-2H2 was diluted in 0.01 M HPES pH 7.4 containing 0.15 M NaCl, 3 mM EDTA and 0.005% P20. All subsequent dilutions were made in the same buffer. All binding experiments were performed at 25°C with concentrations ranging from 0.19 nM to 100 nM at a flow rate of 75  $\mu$ L/min; data were collected for approximately 35 minutes and three 1-minute pulses of 30 mM HCl were used to regenerate the surfaces. Antibodies were also flowed over an uncoated cell and the sensorgrams from these blank runs subtracted from those obtained with rhuIL-9coupled chips. Data were fitted to a 1:1 Langmuir binding model. The algorithm calculates both the  $k_{on}$  and the  $k_{off}$ , from which the apparent equilibrium dissociation constant,  $K_D$ , is deduced as the ratio of the two rate constants ( $k_{off} / k_{on}$ ). These values obtained are indicated in Table 6 below:

**Table 6**

Molecule	$k_{on}$ ( $M^{-1} s^{-1}$ )	$k_{off}$ ( $s^{-1}$ )	$K_D$ (pM)
7F3com-2H2 Fab	$1.68 \times 10^5$	$3.62 \times 10^{-5}$	215
7F3com-2H2 IgG	$4.76 \times 10^5$	$2.65 \times 10^{-6}$	6
MH9A3 Fab	$2.89 \times 10^5$	$1.80 \times 10^{-4}$	623
MH9A3 IgG	$3.02 \times 10^5$	$8.94 \times 10^{-6}$	30



**7.     EQUIVALENTS**

**[00559]**       Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

**[00560]**       All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

## **THE ABSTRACT OF THE INVENTION**

The present invention provides novel antibodies that immunospecifically bind to an IL-9 polypeptide and compositions comprising said antibodies. The present invention also provides methods and compositions preventing, treating, managing, and/or ameliorating diseases and disorders associated with aberrant expression and/or activity of IL 9 or IL-9 receptor or subunits thereof, autoimmune diseases, inflammatory diseases, proliferative diseases, and infections comprising administration of one or more antibodies thereof that immunospecifically bind to an IL-9 polypeptide. The invention also encompasses methods and compositions for diagnosing, monitoring, and prognosing these disorders. The present invention further relates to articles of manufacture and kits comprising antibodies that immunospecifically bind to an IL-9 polypeptide.